Correlation of Herpes Simplex Virus Antibody Titers and Specific Lymphocyte Stimulation in Adult Blood Donors

HANS MOSER,* FRITZ BEHRENS, ROBERT ZIEGELMAIER, JOACHIM HILFENHAUS, AND RUDOLF MAULER

Research Laboratory of Behringwerke AG, 3550 Marburg, Federal Republic of Germany

Antibody titers to herpes simplex virus type 1 in sera from healthy adult donors were assayed by complement fixation, microneutralization, and an enzyme immunoassay (ELISA). This last test proved to be the most sensitive method for antibody detection. It was estimated that ELISA antibody titers were up to 40-fold higher than neutralizing antibody titers and up to 100-fold higher than complement fixation antibody titers. Due to the higher sensitivity of ELISA, only 3 of 36 blood donors tested in this assay were shown to be seronegative, whereas 6 additional persons of the same group were termed seronegative by the microneutralization assay. Furthermore, four of the latter also did not respond in the complement fixation test. In vitro stimulation of peripheral lymphocytes by using a partially purified herpes simplex virus type 1 particle antigen was achieved for all seropositive blood donors. Only those three donors who were ELISA negative reacted negatively in this stimulation assay. From these results it may be concluded that ELISA is an appropriate method not only for rapid and sensitive antibody determination but also for selecting herpes simplex virus-negative patients.

Determination of immunological parameters directed against herpes simplex virus (HSV) plays an important role in routine diagnosis and also in diagnosis of encephalitis or severe systemic diseases of unknown etiology which may be related to HSV infection. In addition, it is of great importance to know about the immunological state of high-risk patients, e.g., renal transplant receivers under immunocompromised conditions, in order to take necessary precautions (5, 13, 19). Furthermore, HSV-specific humoral and cellular immune responses are studied in healthy persons to determine which immunological conditions may be involved in HSV recurrences (8, 9).

Immunity against viral infections can be determined either by specific antibody assays or by specific cell-mediated immune (CMI) response, e.g., by a specific lymphoblast stimulation assay (LSA). In the case of diseases caused by herpesviruses, the CMI response has been shown to play an important role in controlling or eliminating the infections, whereas the humoral immune mechanisms seem to be less effective in this regard (12, 14, 20, 24).

Despite the great importance of CMI response in HSV infections, diagnostic means have usually been limited to serology. This is due to the fact that antibody assays are much easier to perform than the LSA. Moreover, sera can be stored frozen, can be sent anywhere, and can be assayed repeatedly at any time, whereas for CMI assays only freshly prepared blood samples should be used.

We were interested in the correlation of results obtained by antibody assays with those obtained by specific LSA, so we screened a group of normal blood donors for HSV antibody titers by complement fixation (CF) and microneutralization (MN) tests. However, when we compared these results with the results from LSA, we found that a larger number of individuals were seronegative than negative with respect to CMI response. Hence we decided to include the enzyme-linked immunosorbent assay (ELISA) as a more sensitive antibody determination method to further substantiate our results. As we report here, we found an excellent correlation between ELISA and LSA, i.e., the individuals tested were either positive or negative in both assays.

MATERIALS AND METHODS

Antigen preparations. HSV type 1 (HSV-1) strain McIntyre was propagated in U cell monolayer cultures grown in roller bottles. Cells were infected at an input multiplicity of infection of 10^(-3) 50% tissue culture infective doses per cell. When the cultures showed maximal cytopathic effect (2 to 3 days post-infection), the supernatant fluids were collected. After removal of cells and cell debris by low-speed centrifugation, the virus particles were concentrated and purified by continuous-flow ultracentrifugation in a
sucreose gradient (16). Virus peak fractions were pooled, and the specific infectivity was determined. The virus preparation was then diluted 1:50 or 1:100 with an appropriate buffer and heat-inactivated (4 h, 60°C). Control antigen was prepared from supernatant fluids of uninfected cell cultures.

In CF assays, as well as in some ELISA tests, a concentrated lysate of HSV-1-infected HeLa cells was used as antigen. Control antigen was prepared identically from uninfected cells.

**Antibody assays.** CF assay (21) and MN test (23) are classical antibody determination methods which were routinely used. In the MN test, 100% infective doses of HSV-1 strain McIntyre were added to each serum dilution. Two different modifications of ELISA were employed. The first assay was carried out by the method of Bidwell et al. (2, 25). Briefly, partially purified HSV-1 particle antigen in sodium carbonate buffer, goat anti-human immunoglobulin G-peroxidase as conjugate, and o-phenylenediamine plus H2O2 as substrate were used. The optical density was determined at 490 nm. In the second procedure, a concentrated lysate of HSV-1-infected HeLa cells was used as the antigen, an anti-human immunoglobulin G-alkaline phosphatase complex was the conjugate, and p-nitrophenylphosphate was used as the substrate. The optical density of the samples was measured at 405 nm (27; R. Ziegelmaier, F. Behrens, and G. Enders, J. Biol. Stand., in press). In both cases the results were recorded with the TiterTek Multiskan-Photometer (Flow Laboratories GmbH, Bonn, Germany). The antiserum dilution that yielded an optical density exceeding that of the respective control by more than 0.3 units was defined as the HSV-1 antibody titer.

In our experience both the ELISA methods showed the same sensitivity as well as identical antibody titers in HSV-positive sera. In addition, both assay modifications showed the same serum samples to be negative.

**Specific LSA.** Lymphocytes were obtained after centrifugation of defibrinized blood samples on a Ficoll-Hypaque gradient according to the procedure described by Bois et al. (3). The cells were suspended in RPMI 1640 medium supplemented with autologous serum at a concentration of 10^6 cells per ml. After addition of inactivated HSV-1 virions (routine dilutions, containing 10^4, 10^5, and 10^6 50% infective doses before inactivation), the mixture was incubated in microtest plates for 5 days, each well containing 0.2 ml of the cell suspension. The cultures were then labeled for 20 h by addition of 30 nCi of [3H]thymidine per well. Finally, the cells were harvested onto glass fiber filters (Dynatech, Nürtlingen, Germany) by using a Mash II apparatus (Millpore, Neu Isenburg, Germany) and thoroughly washed with water, and the radioactivity was measured in a Beckman liquid scintillation spectrometer (Beckman Instruments GmbH, Munich, Germany).

Stimulation indices were calculated by dividing the mean counts per minute of four cultures incubated with HSV-1 antigen by that of four cultures incubated with a corresponding control antigen. Stimulation indices exceeding 3 were defined as positive.

**RESULTS**

Blood samples from 36 randomly selected donors were assayed for HSV-1 antibodies. At the time of bleeding, none of the volunteers had any clinical manifestations of an acute HSV infection. Antibody titers obtained by CF assay, MN test, and ELISA were compared by plotting the reciprocal values against each other. A comparison of CF and MN antibody titers (Fig. 1) indicated that the MN test gave about fourfold higher titers than the CF assay. However, only seven of nine MN-seronegative donors (of 36 total donors) were also CF antibody negative.

Furthermore, examination of the blood samples by the ELISA method revealed that only three of all the donors lacked HSV-1 antibodies. Six of the nine MN-negative donors, including four of seven CF-negative persons, showed clearly positive antibody titers when tested by ELISA (Fig. 2 and 3). Thus, due to the excellent sensitivity of the latter test (up to 40-fold more sensitive than the MN test and up to 100-fold more sensitive than the CF assay), it was possible to detect those sera which were falsely neg-

![Graph](http://jcm.asm.org/)

**Fig. 1. Comparison of CF versus MN HSV-1 antibody titers.** Mean titers were based on three independent determinations. The dotted lines indicate the limits of sensitivity for the respective antibody determination methods. The broken line represents the theoretical locus of titers assuming equal sensitivity of the two tests.
The 36 adult donors involved in our study could be classified into three groups, as follows. (i) Three donors were seronegative in all three antibody assays, and their lymphocytes could not be stimulated with HSV antigen. Based upon these results, these persons may be regarded as in fact HSV negative (Fig. 4). Moreover, none of these donors had any experience with recurrent HSV infections. (ii) Six donors were found to be seronegative in CF or MN assays or both, but were shown to possess HSV-1 antibodies when tested with the ELISA methods; in addition, their lymphocytes could be specifically stimulated with HSV-1 antigen (Fig. 4). It should be pointed out, however, that the mean stimulation indices in this group were considerably lower than those of the group that was seropositive in all three antibody assays. Interestingly, in this group also none of the donors had suffered from HSV recurrences at any time. (iii) All those donors who were seropositive in the three antibody assays (27 per-

Fig. 2. Comparison of MN versus ELISA HSV-1 antibody titers. For details refer to the legend of Fig. 1.

Active in the other antibody determination methods used in these experiments.

Initially, when we compared the results of HSV-specific stimulation of peripheral lymphocytes derived from fresh blood samples with corresponding MN and CF antibody titers, we discovered a few individuals who exhibited a clearly positive CMI response directed against HSV, even though they were seronegative in both the antibody assays. However, when these sera were studied by ELISA we found complete concordance between antibody titers and LSA (Fig. 4).

As expected, cord blood lymphocytes which were assayed as a control could not be stimulated by HSV-1 antigen (Fig. 4), although they responded normally to the lectins phytohemagglutinin and concanavalin A (data not shown). The cord blood sera contained normal levels of HSV-1 antibodies which presumably were of maternal origin and had been transmitted to the fetus via the placenta.
VOL. 13, recurrent samples antibody region. showed sons) recurrent infections frequently (i.e., of the 60% investigation results was tested were tested responses of 36 namely, It and about up 40-fold. Fig. 3. Comparison of CF versus ELISA HSV-1 antibody titers. Mean titers of sera from cord blood samples (x) and adult donors (O) are plotted.

DISCUSSION
In this report we present the results of an investigation on the HSV-1-specific immune responses of 36 adult blood donors. Blood samples were tested in three different antibody assays, namely, the CF assay, the MN test, and an ELISA method. Furthermore, CMI response was tested by using an HSV-specific LSA. The results indicated that ELISA was the most sensitive and precise of the antibody assays compared. It was estimated that the sensitivity was about 40 times higher than that of the MN test, and up to 100-fold higher than that of the CF assay. The excellent sensitivity of enzyme immunoassays has been previously reported for HSV and other virus systems (2, 10, 11, 17, 18).

The superiority of ELISA over the other antibody determination methods could be demonstrated by comparing the results of antibody determination with the results of HSV-specific lymphoblast transformation: only the lymphocytes from blood donors who proved to be HSV antibody negative in the enzyme immunoassay could not be stimulated by HSV antigen. Thus, ELISA offers an appropriate method for selecting patients who are HSV negative. Furthermore, our study suggests that ELISA yields the most reliable results, since the other antibody determination methods, due to their lower sensitivity, may yield false-negative results. This is an important fact and should be considered in doubtful cases.

Concerning the specificity of our tests, it has previously been reported that neither the LSA nor the ELISA shows cross-reactions between HSV and varicella-zoster virus, cytomegalovirus, or Epstein-Barr virus (1, 6). This was consistent with our finding that the three donors who were HSV-1 antibody negative in the ELISA had high anti-varicella-zoster virus titers, and, in addition, two of them were clearly positive in an anti-cytomegalovirus ELISA (results not shown). However, there is of course considerable cross-reactivity between HSV-1 and HSV-2, as we found by using antisera of rabbits immunized with either HSV-1 or HSV-2 (data not shown). Similar results have been described in the human system by McClung et al. (15).

Furthermore, we observed that more than 50% of the blood donors who were found to be seropositive in all assays suffered frequently from recurrent HSV infection. This is in agreement with the generally accepted fact that normal antibody levels cannot prevent reactivation of HSV (4, 7, 22). The group of donors who were seronegative and did not respond in the lymphocyte transformation assay was considered as unsensitized to HSV. These persons had no history of HSV disease, and it is conceivable that they had never had a primary infection. Interestingly, the group of volunteers with cellular immunity directed against HSV who obviously lacked neutralizing antibodies had never had any signs of herpes recurrence. A possible explanation for this fact might be that these donors had once undergone primary infection, but because of a lack of the booster effect usually caused by recurrent herpes, there was no further stimulation of the immune system. This may also explain the relatively low stimulation indices observed in this group.

In conclusion, ELISA proved to be an excellent method for routine determination of HSV-
1 antibodies, since, as compared to most of the other conventional antibody assays, the test is very easy to perform, precise, rapid, and very sensitive. In those studies which dealt with the investigation of the relationship between humoral and cellular immunity against HSV, the ELISA was certainly advantageous, since its sensitivity was comparable to that of LSA. This sensitivity can help to avoid misinterpretation of results and the drawing of wrong conclusions with respect to the immunological status of a patient.

ACKNOWLEDGMENTS

We are much indebted to J. Troelttsch, Diakonie-Krankenhaus, Marburg, Germany, for providing fresh cord blood samples. The skilful technical assistance of H. Damm, E. Huhn, and K. Schroeder is gratefully acknowledged.

LITERATURE CITED


