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Assay of Type-Specific and Type-Common Antibodies to Herpes Simplex Virus Types 1 and 2 in Human Sera

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A reliable and reproducible method for determining specific reactivity to herpes simplex virus types 1 and 2 (HSV-1 and HSV-2) in human sera has been developed. Human sera were used to immunoprecipitate HSV-specific glycoprotein antigens from both HSV-1- and HSV-2-infected cell extracts. The viral glycoproteins precipitated from these extracts were then analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis to detect specific reactivity of the sera with distinct type-specific antigens of HSV-1, HSV-2, or both as well as with type-common glycoprotein antigens. By examining a large number of human sera, this method was found to be more reliable than the standard microneutralization test in discriminating between single-positive (positive for HSV-1 or HSV-2) and double-positive (positive for HSV-1 and HSV-2) sera.

Over a decade ago, an association between prior genital herpes simplex virus type 2 (HSV-2) infections and subsequent development of cervical carcinoma in women was independently reported by Rawls et al. (23) and Josey et al. (9). Despite the technical difficulties in identifying sera having specific anti-HSV-2 activity, this correlation has since been confirmed and extended by a number of other investigators using various techniques (1, 7, 8, 15, 25). However, not all women experiencing genital HSV-2 infection develop cervical carcinoma later in life. Assuming HSV-2 to be the causative agent (an assumption which will be difficult to prove conclusively), there are various ancillary factors which may also affect the risk of an individual's developing cervical carcinoma. These include (i) the timing of the primary genital herpes infection relative to the developmental differentiation of the uterine cervix, (ii) the presence or absence of any possible cofactors which may also play a role in the induction of the carcinoma, and (iii) previous infection of the individual with HSV-1 (nongenitally), which has the effect of lessening the severity of subsequent HSV-2 infections (A. J. Nahmias et al., presented at Fourth International Congress for Virology, The Hague, Netherlands, 1978). Definitive investigations into the possible protective effect of a previous infection with HSV-1 will, however, require methods of serotyping which can reliably distinguish between single-positive (HSV-1 or HSV-2) and double-positive (HSV-1 and HSV-2) sera.

Several techniques have been applied to the problem of differentiating human sera reactive with HSV-1, HSV-2, or both (reviewed in reference 19). Among these are the microneutralization test with values expressed as either a II/I index (22) or a pN (potency of neutralization) value (11), neutralization kinetic tests (28), fluorescent-antibody tests (30), antibody-complement cytolysis (29), and hemagglutination inhibition assays (25). One limitation of all of these tests is that antibodies to a number of undefined viral antigens, both type common and type specific, are assayed simultaneously. Thus, although antibodies directed against type-specific antigenic sites may be measured, the type-specific reaction may well be overshadowed by the simultaneous assay of type-common antigenic sites.

One approach to dealing with this problem has been to cross-adsorb the sample sera before testing in radioimmunoassays, using crude infected cell lysates as the antigen (6, 16). An additional approach has been to purify certain type-specific antigens of HSV-1 and HSV-2 and use these in a sensitive radioimmunoassay test (3). Although sensitive, such approaches do require considerable time and expense in preparing the antigens and antisera used. In addition, such tests using a single antigen inherently assume that all positive sera will have reactivity to the specific antigens used. The most serious drawback to such an assumption is that antigenic variation observed among different HSV strains (26) may, at least in part, be due to antigenic variation of the particular antigen(s) in question. In addition, the antibody levels to individual antigens may vary among sera, which could lead to an aberrant diagnosis when such is made on the basis of a serum's reactivity with a single antigen.

The presence of HSV-specific glycoproteins

on the external surface of both virions (14) and infected cells (17) implies that these antigens are accessible to the immune system of the infected host. Accordingly, it has been shown that the viral glycoproteins represent the targets of such varied immune response mechanisms as virus neutralization (20, 27), antibody-dependent cellular and complement-mediated cytolysis (12), and T-cell-mediated cytotoxicity (10). Since certain glycoproteins of HSV-1 and HSV-2 are known to be type specific and others type common, we examined the reactivity of various immune sera with individual glycoprotein antigens of HSV-1 and HSV-2 as a possible means of typing human sera.

MATERIALS AND METHODS

Cells and tissue culture. HEp-2 and Vero cells were propagated in Eagle minimal medium supplemented with 10% donor calf serum, 100 IU of penicillin per ml, 100 μg of streptomycin per ml, and 0.075% $NaHCO_3$. HEp-2 cells used to prepare viral antigen were grown in 100-mm plastic petri dishes in the same medium containing 0.225% $NaHCO_3$ and kept in a humidified, 5% CO_2 atmosphere. After infection of cells, all cultures were maintained in Eagle minimal medium containing 2% donor calf serum, 100 IU of penicillin per ml, 100 μg of streptomycin per ml, and 0.225% $NaHCO_3$ (maintenance medium). MRC5 cells (human embryonic lung) were used to prepare all virus stocks. This cell line was propagated in the same medium used for HEp-2 cells except that fetal calf serum was used instead of donor calf serum.

Viruses. The HSV-1 strain designated BK [HSV-1(BK)] was used throughout. This virus strain was isolated from a recurrent lip lesion and was passed four times in MRC5 cells before use. This strain was typed as an HSV-1 serotype on the basis of (i) neutralization tests and (ii) the polypeptide profile of infected cells. Studies conducted on HSV-1(BK) have indicated that the glycoproteins of this strain are identical in both their electrophoretic mobility on sodium dodecyl sulfate (SDS)-polyacrylamide gels and their antigenic properties to the established KOS strain. The 186 strain of HSV-2 [HSV-2(186)] was also used.

Preparation of infected cell extracts. Semiconfluent HEp-2 cell monolayers were infected with either HSV-1(BK) or HSV-2(186) at a multiplicity of infection of 10 plaque-forming units/cell. The viral inoculum was absorbed for 1 h at 37°C, after which the inoculum was removed, 3 ml of maintenance medium was added, and the cultures were incubated for an additional 24 h at 37°C. Four hours after infection, D-[1-^{14}C]glucosamine (specific activity, 61 mCi/mmol; Amersham Corp., Arlington Heights, Ill.) was added to a final concentration of 3.3 μCi/ml. At 24 h postinfection, cells were scraped into the medium, pelleted, and washed three times with phosphate-buffered saline. Cell pellets were suspended in distilled water at approximately 2×10^7 cells/ml, sonicated for 2 min, freeze-thawed, and adjusted to 1% sodium deoxycholate and 1% Tween 40, using 10% solutions of each detergent. After thorough mixing, lysates were incubated at 4°C for 1 h and centrifuged at 100,000 × *g* for 1 h to pellet insoluble material. The supernatants were removed, put into plastic test tubes (12 by 75 mm) in 25-μl amounts, and frozen at −20°C until needed. Single preparations of HSV-1- and HSV-2-infected cell extracts were used in all experiments.

Sera. Hyperimmune rabbit antisera to HSV-1- and HSV-2-infected RK13 cells were prepared by intramuscular injection of Formalin-inactivated infected cell lysates followed by repeated injections of noninactivated infected cell lysates. Human sera obtained in this laboratory were drawn by certified personnel from voluntary, informed donors. Blood was allowed to clot for 12 to 18 h at 4°C, and the serum was removed, diluted into equal portions, and stored at −20°C. Additional human sera were kindly provided under code by Ervin Adam, Department of Virology and Epidemiology, Baylor College of Medicine, Houston, Tex.

Immunoprecipitation assays. Each serum was tested simultaneously against both HSV-1 and HSV-2 antigen preparations. To each tube of antigen was added 100 μl of TNE-TX [0.05 M tris(hydroxymethyl)aminomethane (Tris), 0.15 M NaCl, and 0.005 M ethylenediamine tetraacetic acid, pH 7.4, containing 0.1% Triton X-100] and 25 μl of serum. Samples were mixed and incubated at 37°C for 1 h and for an additional 6 to 8 h at 4°C. Staphylococcus A (Calbiochem-Behring Corp., La Jolla, Calif.) was then added (20 μl) and incubated for an additional 18 to 24 h at 4°C to precipitate antigen-antibody complexes from solution. Immune precipitates were washed three times with TNE-TX and solubilized at 37°C in 25 μl of 2% SDS, 2% β-mercaptoethanol, and 1 M urea in 0.05 M Tris-hydrochloride (pH 6.7) containing 4% sucrose and bromophenol blue.

SDS-PAGE. Procedures used in analytical slab SDS-polyacrylamide gel electrophoresis (PAGE) have been described previously (5, 21). All gels were 8.6% acrylamide cross-linked with *N,N'*-diallyltartardiamide. Immediately before electrophoresis, all samples were heated at 100°C for 1 min in 2% SDS, 2% β-mercaptoethanol, and 1 M urea in 0.05 Tris-hydrochloride (pH 6.7). All gels were electrophoresed at 15 mA/gel for a total distance of 9.5 cm. Gels were dried under vacuum and exposed to Kodak NS2T X-ray film for detection of radiolabeled polypeptides.

Microneutralization assay. Assays of human sera for virus-neutralizing activity were performed by using the microneutralization test essentially as described previously by Pauls and Dowdle (18). Virus stocks of HSV-1(BK) and HSV-2(186) were diluted to 4×10^6 plaque-forming units/ml and frozen in equal portions at −80°C. Neutralization was carried out by combining 100 μl of a 1:10 dilution of either virus stock with 100 μl of the test serum serially diluted twofold in individual wells of 96-well U-bottom microtiter plates. Virus-serum mixtures were mixed and incubated at 37°C for 1 h, after which 100 μl of each sample was overlayed onto Vero cell monolayers grown in 96-well flat-bottom microtiter plates. After 4 days, wells containing serum dilutions which showed greater than 50% destruction of the indicator cell monolayer were considered negative for neutralizing activity, and dilutions exhibiting less than 50% destruction of the cell mono-

layer were considered positive for neutralizing activity. Determination of the presence of anti-HSV-2 activity in sera exhibiting neutralization of HSV-1 was made by using the II/I index as described by Rawls et al. (22). The II/I index was calculated as follows:

$$\frac{\log_{10} \text{titer of serum versus HSV-2}}{\log_{10} \text{titer of serum versus HSV-1}} \times 100 = \text{II/I index}$$

Sera exhibiting a II/I index of greater than 85 were considered positive for HSV-2, whereas sera having II/I index of less than 85 were considered negative for HSV-2.

RESULTS

Figure 1 shows glycoproteins induced in cells infected by HSV-1 or HSV-2 which have been antigenically characterized. The major glycoprotein regions of both serotypes (average molecular weight, 125,000 to 130,000) were composed of three readily discernible glycoproteins designated gA, gB, and gC. Evidence has been presented elsewhere that gA and gB represent different forms of the same polypeptide in HSV-1 and probably in HSV-2 as well (4). These two glycoproteins reacted predominantly as type-common antigens, although they had type-specific determinants as well. The second antigen of the major glycoprotein region was the gC glycoprotein (1gC and 2gC). These two glycoproteins represent type-specific antigens, since no detectable cross-reactivity exists between 1gC and 2gC (R. Eberle and R. J. Courtney, *in:* International Conference on Human Herpesviruses, in press). The second prominent region of the HSV-specific glycoproteins had an average molecular weight of approximately 55,000 to 60,000. Of the two glycoproteins visible in extracts of infected cells, only one was readily detectable in immune precipitates obtained by using human immune sera. These glycoproteins (1gD and 2gD) react in a type-common manner (2, 27, 32). Another glycoprotein region present in HSV-2-infected cells which is relevant to this study had an average molecular weight of 35,000 to 50,000 and consisted of three to four glucosamine-labeled bands. By using specific antisera prepared to the individual glycoproteins of the major glycoprotein region (Eberle and Courtney, submitted for publication), these glycoproteins did not appear to be antigenically related to any

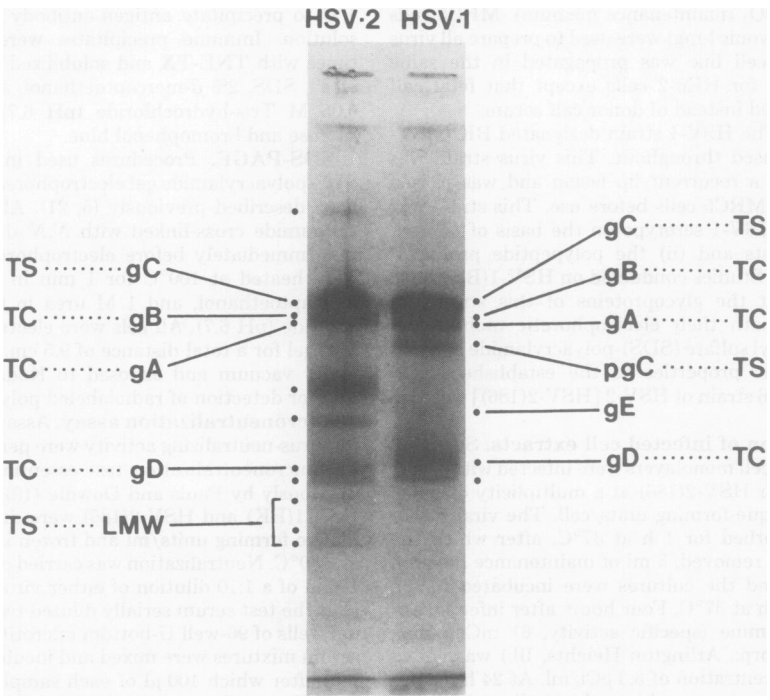

FIG. 1. *Identification of HSV-1- and HSV-2-specific glycoproteins. HEp-2 cells were infected with HSV-1(BK) or HSV-2(186) and labeled with [^{14}C]glucosamine from 4 to 24 h postinfection. Detergent extracts of HSV-1 and HSV-2 were prepared and analyzed by SDS-PAGE as described in Materials and Methods. All of the virus-specific glycoproteins detected are indicated (●). The antigenic properties of each of the glycoproteins relevant to this study are also indicated (type common, TC; type specific, TS).*

of the virus-specific glycoproteins of higher molecular weight. As no accepted nomenclature has as yet been applied to these glycoproteins, they will be referred to here simply as the LMW (low molecular weight) glycoproteins. By immunoprecipitation tests, the LMW glycoproteins reacted as type-specific antigens. Other glycoproteins readily observed in infected cell extracts which will not be dealt with in detail here included the 1pgC glycoprotein, which is a precursor of the 1gC glycoprotein and as such reacts type specifically, the 1gE glycoprotein, and two HSV-2 glycoproteins of similar molecular weight. Although prominent in infected cell extracts, these glycoproteins were not found in appreciable quantities in immune precipitates obtained with human sera.

Immunoprecipitation of viral glycoproteins by hyperimmune rabbit sera. To determine the feasibility of typing immune sera on the basis of the glycoproteins that they immunoprecipitate from HSV-infected cell extracts, sera prepared in rabbits to HSV-1 and to HSV-2 were initially tested. Since these sera were of known specificity (anti-HSV-1 or anti-HSV-2), they should have clearly discriminated between type-specific and type-common glycoprotein antigens, thereby permitting confirmation of previous antigenic analyses of HSV glycoproteins to be made by the immunoprecipitation-gel analysis (IPGA) system. Figure 2 shows an SDS-PAGE of immune precipitates from HSV-1- and HSV-2-infected cell extracts by immune and nonimmune rabbit sera. The normal rabbit serum did not specifically precipitate any of the virus-specific glycoproteins from infected cell extracts. By using the anti-HSV-1 and anti-HSV-2 sera, the gA, gB, gA/gB dimers (the highest-molecular-weight species identified in Fig. 2), and gD glycoproteins were precipitated from both HSV-1- and HSV-2-infected cell extracts, thereby confirming the presence of type-common antigenic sites on these three glycoproteins. The two prominent glycoproteins of HSV-2 which migrated between 2gA and 2gD also appeared to have at least some type-common determinants, since these were precipitated by anti-HSV-1 sera, albeit inefficiently. In contrast, the gC glycoproteins were precipitated only from the homologous infected cell extracts, indicating their type-specific antigenicity. Similarly, a glycoprotein antigenically related to 2gC (Eberle and Courtney, unpublished data) but exhibiting a significantly greater apparent molecular weight was precipitated from HSV-2-infected cell extracts only by anti-HSV-2 sera. The LMW glycoproteins of HSV-2 were not effectively precipitated by any of the rabbit antisera examined. Thus, reaction of a serum with the 1gC or 2gC glycoprotein may be taken as indicating the presence of specific anti-HSV-1 or anti-HSV-2 activity in that serum, respectively.

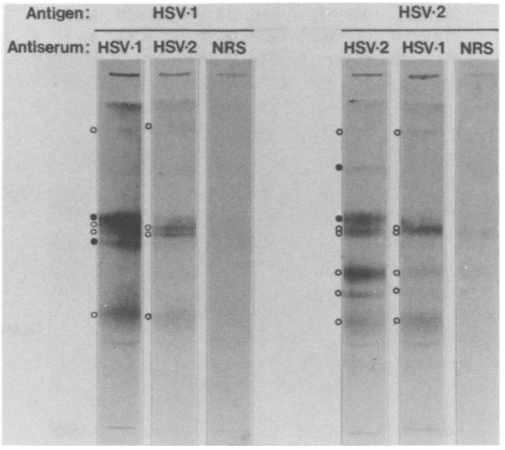

FIG. 2. *Immunoprecipitation of HSV glycoproteins by hyperimmune rabbit sera. Antisera prepared in rabbits to HSV-1- or to HSV-2-infected cells or normal rabbit sera were reacted with HSV-1- and HSV-2-infected cell extracts. Immune precipitates were then analyzed by SDS-PAGE. Type-common* (○) *and type-specific* (●) *glycoproteins present in the immune precipitates are identified. All samples were run on the same gel but have been rearranged for presentation.*

Immunoprecipitation of HSV-specific glycoproteins by human sera. Human sera of known reactivity to each HSV serotype were used initially to determine the patterns of precipitation of HSV glycoproteins obtained with human sera. For this purpose, HSV-1-positive, HSV-2-negative (+/−), HSV-1-negative, HSV-2-positive (−/+), and double-negative (−/−) sera were tested for their ability to specifically immunoprecipitate [^{14}C]glucosamine-labeled glycoproteins from extracts of HSV-1- and HSV-2-infected HEp-2 cells (Fig. 3). Nonspecific background levels of precipitation of HSV-1 or HSV-2 glycoproteins obtained using the IPGA assay were defined as the amounts detected by using (−/−) sera. HS-11, a representative (+/−) serum, specifically precipitated all of the major glycoproteins observed in HSV-1-infected cell extracts. However, only the type-common glycoproteins (2gA, 2gB, and 2gD) were precipitated from HSV-2-infected cell extracts by (+/−) sera; neither the 2gC nor the LMW glycoproteins were ever specifically immunoprecipitated by any (+/−) sera. A similar pattern of reactivity was observed with (−/+) sera in that essentially all of the HSV-2 glycoproteins and the type-common HSV-1 glycoproteins (1gA, 1gB, and 1gD) were specifically precipitated. The type-

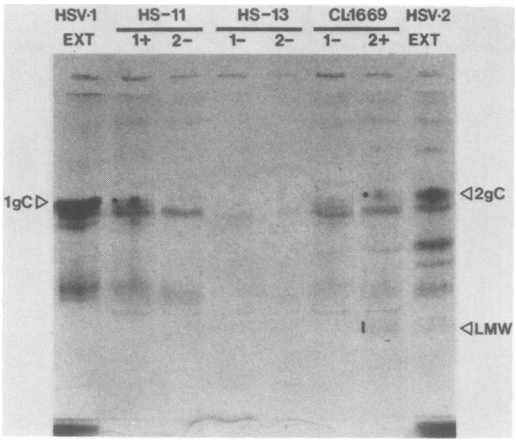

FIG. 3. *Immunoprecipitation of HSV glycoproteins by human sera. Human sera from individuals infected with only HSV-1 (HS-11), only HSV-2 (CL-1669), or neither HSV serotype (HS-13) were reacted with HSV-1- and HSV-2-infected cell lysates, and the immune precipitates were analyzed by SDS-PAGE. The major type-specific glycoproteins of HSV-1 (1gC) and HSV-2 (2gC, LMW) are identified in the infected cell lysates. Specific precipitation of these glycoproteins by the sera is indicated* (●).

specific 1gC and 1pgC glycoproteins were not specifically precipitated by any of the (−/+) sera examined. These results suggest that the specific precipitation of the gC glycoprotein of either HSV serotype indicates a past infection by that serotype. Precipitation of the type-specific LMW glycoproteins from HSV-2-infected cell extracts by human sera provided further evidence of specific anti-HSV-2 activity in sera.

During these studies, we noted that a number of HSV-2-reactive sera did not precipitate HSV-2 glycoproteins as well as HSV-1-reactive sera precipitated HSV-1 antigens. This, coupled with the diffuse nature of the 2gC glycoprotein on SDS-polyacrylamide gels, made specific precipitation of this glycoprotein sometimes difficult to distinguish from nonspecific immunoprecipitation of the 2gC glycoprotein. However, the LMW glycoproteins of HSV-2 were always observed in immune precipitates of HSV-2-reactive sera but never in immune precipitates of (−/−) or (+/−) sera. Therefore, the LMW glycoproteins actually served as a better indicator of specific HSV-2 activity in sera than did the 2gC glycoprotein. Further investigation into the nature of the glycoproteins which comprise the LMW glycoprotein region is currently underway.

Analysis of coded human sera by the IPGA assay. Having established the feasibility of differentiating HSV-1 and HSV-2 activity in sera by the IPGA test, it was necessary to determine the reliability and sensitivity of this method. For this purpose, 16 coded human sera and a number of uncoded sera collected in this laboratory were analyzed by the IPGA procedure (Fig. 4). All sera which specifically precipitated the 1gC glycoproteins from HSV-1-infected cell extracts were considered positive for HSV-1. Similarly, all sera which exhibited definite precipitation of the 2gC glycoprotein, the LMW glycoproteins, or both from HSV-2-infected cell lysates were considered to have specific anti-HSV-2 activity. Recognition of all three of these type-specific glycoproteins (1gC, 2gC, and LMW glycoproteins) was the criterion used to identify a serum as (+/+). Sera exhibiting no specific precipitation of any of the HSV glycoproteins were considered (−/−).

For comparative purposes, each serum was also typed by microneutralization. This test can readily distinguish HSV-negative from HSV-positive sera. In addition, by testing each serum for its relative ability to neutralize HSV-1 and HSV-2 and by using the statistically derived II/I index (22), the presence of specific anti-HSV-2 activity in sera can usually be detected. However, this index does not serve to distinguish been (+/+) and (−/+) sera. Table 1 presents the results of microneutralization tests on each of the coded sera and many of the sera collected in this laboratory, together with the results obtained with the IPGA assay. For all but four sera, the serotypes as determined by each method were compatible. In two of these four cases (sera CL-1707 and G-698), the IPGA assay indicated specific anti-HSV-2 activity, whereas the microneutralization test did not. This was probably due to masking of anti-HSV-2 neutralizing activity by high levels of activity in these sera directed against cross-reactive HSV-1 antigens. Another serum (HS-15) had a II/I index of 89 and was therefore considered to have specific anti-HSV-2 activity by the microneutralization test, whereas the IPGA assay indicated anti-HSV-1 activity only. This serum had a very high neutralizing titer relative to the other sera tested and was obtained from an individual who suffered frequent recurrent lip lesions. Thus, the false indication of specific HSV-2 activity in this serum by the microneutralization test may have been due to the presence of more antibodies which were reactive with type-common rather than type-specific antigenic determinants of the virus. The fourth serum (HS-16) was obtained from an individual who had no history of oral or genital herpes infections. This serum was typed as HSV positive by the microneutralization test at both 1:10 and 1:20 dilutions. Analysis of this

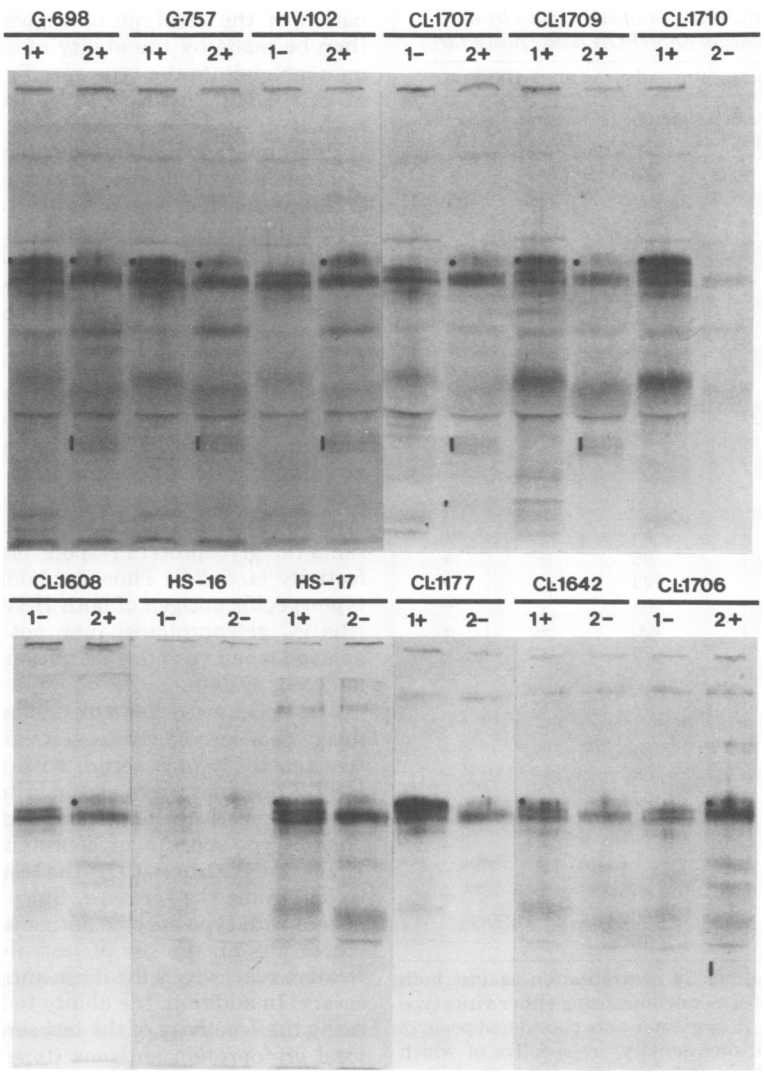

FIG. 4. *Immunoprecipitation of HSV glycoproteins by representative human sera. Sera were reacted with both HSV-1- and HSV-2-infected cell extracts, and the immune precipitates were analyzed by SDS-PAGE. Specific precipitation of the 1gC, 2gC, or LMW glycoproteins by the sera is indicated* (●). *The serotype arrived at for each serum is also presented immediately below the serum designations.*

serum by the IPGA assay indicated that this serum was actually HSV negative (see Fig. 4). Therefore, this serum apparently had some activity which nonspecifically inactivated both HSV-1 and HSV-2 up to a 1:20 dilution of the serum, thereby resulting in a false-positive diagnosis for HSV by microneutralization analysis.

DISCUSSION

The immunological analysis in recent years of many of the major glycoproteins of HSV-1 and, to a lesser extent, HSV-2 has resulted in the positive identification of one type-specific glycoprotein (gC) induced in cells infected by each HSV serotype (13, 31; Eberle and Courtney, in press). In addition, several glycoproteins of both HSV serotypes (gA, gB, and gD) possess type-common antigenic sites (2, 27, 31, 32; Eberle and Courtney, in press). All of the corresponding glycoproteins (e.g., all gC glycoproteins) of various HSV strains within a given HSV serotype appear to have enough antigenic determinants in common that antibodies elicited by one virus strain will react with the same glycoprotein of other HSV strains within that serotype (4;

TABLE 1. *Correlation of serotyping by the microneutralization and IPGA assay procedures*

Serum no.	Microneutralization		IPGA	
	Anti-HSV[a]	II/I index[b]	Anti-HSV-1	Anti-HSV-2
CL-1177	+	81	+	−
CL-1334	+	100	+	+
CL-1601	+	52	+	−
CL-1607	+	68	+	−
CL-1608	+	100	−	+
CL-1612	+	100	−	+
CL-1617	−		−	−
CL-1642	+	79	+	−
CL-1669	+	114	−	+
CL-1706	+	116	−	+
CL-1707[c]	+	53	−	+
CL-1709	+	68	+	−
CL-1710	+	76	+	−
CL-1711	−		−	−
CL-1712	−		−	−
G-698[c]	+	59	+	+
G-757	+	86	+	+
HV-102	+	123	−	+
HS-1	+	68	+	−
HS-2	+	100	+	+
HS-3	+	73	+	−
HS-4	−		−	−
HS-5	+	57	+	−
HS-6	−		−	−
HS-7	+	64	+	−
HS-9	+	68	+	−
HS-10	+	57	+	−
HS-11	+	68	+	−
HS-12	−		−	−
HS-13	−		−	−
HS-15[c]	+	89	+	−
HS-16[c]	+	100	−	−
HS-17	+	76	+	−

[a] Sera were tested by neutralization against both HSV-1 and HSV-2. Sera neutralizing either virus type at a dilution of 1:10 or greater were considered positive for HSV neutralizing activity, irrespective of which HSV serotype this activity was directed against.

[b] Calculated as described in Materials and Methods. A II/I index greater than 85 was considered indicative of neutralizing activity specifically directed against HSV-2 in that serum. This index does not signify the presence or absence of HSV-1 neutralizing activity.

[c] Serum for which the serotypes obtained by microneutralization and IGPA did not correlate with one another.

Eberle and Courtney, unpublished data). This, together with the known antigenic reactivity of each of these glycoproteins and the ability to resolve and identify each on SDS-polyacrylamide gels, has permitted the development of the IPGA serotyping assay described here. Thus, the specific reactivity of human sera with individual glycoprotein antigens of HSV-1 and HSV-2 can be determined by analyzing immune precipitates on SDS-polyacrylamide gels. Determination of the serotype of a given serum may then be made by the ability of a serum to immunoprecipitate the type-specific glycoproteins of HSV-1 (1gC) or HSV-2 (2gC and LMW), or both.

The simultaneous but indistinguishable assay of antibodies of all specificities (type common and type specific) in most of the assay systems used to date has made differentiation of HSV-1-positive, HSV-2-positive, and double-positive sera difficult. In tests relying on virus neutralization for antibody detection, this problem is further compounded by the fact that only antibodies capable of neutralizing the virus are measured. Within the major glycoprotein region of both HSV-1 and HSV-2 (average molecular weight, 125,000 to 130,000), the type-specific gC glycoprotein does not appear to play a major role in neutralization of the virus. Rather, the type-common gB glycoprotein antigen represents the glycoprotein responsible for virion infectivity (4, 5, 24). Thus, antibodies to a major type-specific antigen of both HSV-1 and HSV-2 (the gC glycoproteins) may not be efficiently assayed when viral neutralization is used as the indicator system.

The IPGA assay has a distinct advantage over most other serodiagnostic tests in that the relative reactivity of a serum with HSV-1 versus HSV-2 does not serve as the criterion for serotype diagnosis. Rather, the recognition of individual type-specific glycoprotein antigens of HSV-1, HSV-2, or both by the test serum is used to determine the serotype. Since the reactivity of sera with type-specific antigens is either present or absent, the use of statistical analysis of relative reactivity with these antigens is unnecessary. In addition, the ability to independently assay the reactivity of the test serum with other viral glycoprotein antigens (type common and type specific) may further corroborate the serotype. Thus, the resulting identification of sera as positive or negative for HSV-1, HSV-2, or both by the IPGA technique may prove to be more reliable than results obtained with other techniques. This is particularly evident when human sera are typed by the IPGA and microneutralization procedures. Although the tests agree well on whether specific anti-HSV-2 activity is present in the sera, with the microneutralization test it is not possible to distinguish between sera which are positive for only HSV-2 and those which are positive for both HSV-1 and HSV-2. Such sera are, however, readily identifiable by the IPGA assay.

At present, the IPGA assay as described here is in no way meant to be quantitative. In addition, the purpose of this method is not to differentiate sera on the basis of the relative levels of

their reactivity with the various viral glycoproteins. Rather, this assay is intended to serve as a reliable method for determining the presence or absence of activity in serum samples which is directed against the various type-specific antigens of one or both HSV serotypes. Quantitative determination of antibody levels to specific glycoprotein antigens of the two HSV serotypes would be more readily obtained by using techniques of greater sensitivity such as radioimmunoassays.

One advantage of this test is the relative simplicity of the system in that no unusual or expensive equipment or complex techniques are required to perform this test. Although not suited to routine diagnosis of HSV infections in humans due to the time required for autoradiographic exposure of X-ray films, the unambiguous results obtained with the IPGA test may prove to be the most desirable means of typing human sera in research-oriented epidemiological and immunological studies of human HSV infections.

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