

Solid Phase Radioimmunoassay for Typing Herpes Simplex Viral Antibodies in Human Sera

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An indirect solid phase radioimmunoassay (RIA) was developed for typing antibody to herpes simplex virus (HSV) types 1 and 2 (HSV-1 and HSV-2) in human sera. The test is based upon absorption of sera with uninfected, HSV-1-infected, and HSV-2-infected cells and testing for residual antibody. The high sensitivity of the RIA method for detecting HSV antibody permits examination of sera at high dilutions, and thus relatively small volumes of virus-infected cells are required for cross-absorption of antibodies. Results obtained in RIA typing of HSV antibodies showed good agreement with those obtained by microneutralization and inhibition of passive hemagglutination. The HSV antibody type(s) determined by RIA also showed good correlation with the virus type isolated from the individual, either from clinical specimens or sensory nerve ganglia. The technique was very sensitive for detection and typing of HSV antibodies in cerebrospinal fluids. The RIA method was highly suitable for detecting two types of HSV antibody in the same serum specimen, and it was possible to show that a marked, type-specific antibody response to HSV-2 does occur in individuals with a primary HSV-2 infection who have experienced a prior infection with HSV-1.

A variety of techniques has been described for type-specific identification of herpes simplex virus (HSV) antibodies in human sera (14), but most have had significant limitations, either because of inability to definitively identify antibodies against both virus types in the same serum or because they are too cumbersome or costly for routine use. Methods such as indirect fluorescent antibody staining or inhibition of indirect hemagglutination, for which sera are cross-absorbed with virus-infected cells and then examined for residual, type-specific antibody, have been useful but they require relatively large amounts of virus-infected cells for absorption.

Radioimmunoassay (RIA) procedures have generally been found to be much more sensitive than conventional serological methods such as neutralization, complement fixation, or fluorescent antibody staining for detection of viral antigens and antibodies (8, 9, 11, 16, 18), and an indirect solid phase RIA method was developed in this laboratory for typing HSV in clinical materials which was more effective and sensitive than direct fluorescent antibody staining (7). A solid phase RIA procedure has subsequently been developed for type-specific identification of HSV antibodies in human sera. It is based upon absorption of sera with uninfected, HSV type 1 (HSV-1)-infected, and HSV type 2

(HSV-2)-infected cells and testing for residual antibody by the indirect RIA method. This report describes the development and evaluation of the RIA method for typing HSV antibody in human sera.

MATERIALS AND METHODS

Virus-infected cell cultures. The MacIntyre strain of HSV-1 and the MS strain of HSV-2 were employed throughout these studies. HSV-infected cell cultures in the bottom of 1-dram vials were utilized in the RIA test. Cultures were established by adding 1 ml of a suspension of human fetal diploid lung (HFDL) cells containing approximately 50,000 cells to each glass vial; growth medium consisted of 10% fetal bovine serum and 90% Eagle minimal essential medium prepared in Earle balanced salt solution. After 2 to 3 days of incubation at 36 C the cultures contained approximately 100,000 cells, and they were infected, without a medium change, with HSV-1 at a ratio of approximately 0.3 infectious particles per cell or with HSV-2 at a ratio of approximately 0.1 infectious particles per cell. After incubation at 35 C for 20 h for HSV-1 or 15 h for HSV-2, the cultures showed a 3-plus viral cytopathic effect, and fluorescent antibody staining indicated that at least 90% of the cells were infected. The medium was removed, and the cells were rinsed with 1 ml of distilled water. Without drying, the cells were fixed with acetone for 20 min. The fixed cultures could be stored at -70 C indefinitely.

Virus-infected cells for absorption of test sera

were prepared by inoculating HFDL cell cultures in roller bottles with HSV-1 or HSV-2 at a multiplicity of ≥ 1 infectious particles per cell and harvesting 2 to 3 days later when the cultures showed a 3- or 4-plus specific viral cytopathic effect. Uninfected cell cultures were prepared and harvested in parallel. Cells were dislodged from the bottle surface by shaking with glass beads, sedimented by centrifugation at 1,500 rpm for 5 min, and then washed once with 0.01 M phosphate-buffered saline, pH 7.2 (PBS), by centrifugation at 1,500 rpm for 5 min. After aspiration of the wash fluids, the cell packs were stored at -70°C .

Sera and CSF examined. Antisera to the MacIntyre strain of HSV-1 and MS strain of HSV-2 produced in rabbits or hamsters (1) were employed as reference sera for the development of the RIA antibody typing procedure. The type specificity in the indirect RIA system of these sera after absorption with heterotypic virus-infected cells has been described previously (7). The RIA method for typing HSV antibody in human sera was evaluated on several panels of human sera. These included 9 coded sera from A. J. Nahmias, on which he had determined the HSV antibody type(s) using the microneutralization technique (13); a group of 37 sera collected by Richard Baringer, Veterans Administration Hospital, San Francisco, Calif., from individuals on whom isolation of HSV had been attempted at autopsy from trigeminal and sacral sensory ganglia (3, 4); and sera from our diagnostic files on patients from whom HSV had been isolated from clinical specimens. Four sera were also examined in which the HSV antibody had previously been typed by inhibition of the indirect hemagglutination reaction (2, 5).

HSV antibody typing by the RIA method was also done on sera or cerebrospinal fluids (CSF) from certain patients with clinical diagnoses of HSV infection on whom virus isolation attempts were either not done or were negative, but who showed fourfold or greater increases in complement-fixing antibody to HSV.

Absorption of test sera and CSF with virus-infected and uninfected cells. For routine testing, each serum diluted 1:1,000 in PBS was added in volumes of 1.2 ml to approximately $10\text{-}\mu\text{l}$ volumes of packed (i) uninfected, (ii) HSV-1-infected, and (iii) HSV-2-infected cells. This gave a ratio of roughly 10 volumes of packed cells to 1 volume of undiluted serum. For the preliminary experiments conducted to determine the optimal concentration of cells to use for absorption, sera diluted 1:1,000 were treated with various volumes of packed cells which gave ratios of packed cells to undiluted serum ranging from 2.5 to 20. The serum-cell mixtures were shaken and incubated at 37°C for 30 min, followed by overnight incubation at 4°C with constant shaking. After centrifugation at $80,000 \times g$ for 1 h, each of the three samples of absorbed serum was removed and diluted appropriately (see below) for examination for residual antibody. CSF was absorbed in the same manner, but at a 1:100 dilution.

Purification and radiolabeling of anti-human globulins. Antiserum produced in goats against human gamma globulin was obtained from Antibodies

Inc., Davis, Calif. The serum gave a single line of precipitate in the immunoglobulin G (IgG) region in immunoelectrophoresis reactions against whole human serum. The gamma globulin was precipitated from the serum three times by $1/3$ saturation with ammonium sulfate and then passed through a diethylaminoethyl-cellulose column. The protein concentration of the purified IgG was determined by both the biuret and Lowry methods.

The purified IgG was labeled with ^{125}I by the chloramine T method (10, 12); ^{125}I in carrier-free form (New England Nuclear) was added at a concentration of 1 mCi/mg of protein (6). The residual, unbound ^{125}I was removed by passing the preparation through a P-B-1 ion exchange column (E. R. Squibb and Sons, New York, N.Y.), and the labeled protein was then dialyzed at 4°C for 4 days against eight changes of PBS, pH 7.0. Precipitation with 20% trichloroacetic acid indicated that over 99% of the radioactivity in the dialyzed preparation was bound to the purified IgG. Assuming 100% recovery of labeled IgG, the specific radioactivity was approximately $0.5 \mu\text{Ci}/\mu\text{g}$ of protein. To protect against loss of ^{125}I -labeled IgG during column purification and dialysis, normal goat serum was added at a concentration of 5% as a carrier protein; therefore a protein determination was not done on the preparation after removal of free ^{125}I .

On the basis of experiments described previously (7), the labeled immunoglobulins were diluted in PBS containing 5% normal goat serum to contain approximately 40,000 counts/min in a volume of 0.1 for use in the indirect RIA procedure. It was found that using a constant amount of radioactivity gave more consistent and reproducible results with a given labeled antibody preparation over a period of 3 to 4 months than did the use of a constant concentration of gamma globulin, due to decay of radioactivity.

Solid-phase RIA procedure for typing HSV antibody. Each of the three portions of absorbed serum diluted 1:1,000 or 1:2,000 in PBS was tested in duplicate against uninfected, HSV-1-infected, and HSV-2-infected cells. Volumes (0.2 ml) of absorbed sera were added to uninfected and virus-infected cell cultures in 1-dram vials, and the tests were incubated overnight at room temperature. The contents of the vials were then aspirated, and the vials were washed two times with 3 ml of PBS. The ^{125}I -labeled anti-human globulin, diluted in PBS with 5% normal goat serum to contain approximately 40,000 counts/min in a volume of 0.1 ml, was added to the vials in a volume of 0.1 ml with a Micropipette Dispenser (Centaur Chemical Co.), and incubation was conducted at room temperature for 70 to 80 min. The contents of the vials were aspirated, and the vials were washed three times with 3 ml of PBS. Residual radioactivity was assayed directly in the vials with a gamma counter (Abbott Logic series counting system, model 111-A, or Beckman Gamma 300 Radiation Counter).

Counts per minute obtained in the duplicate tests were averaged, and binding ratios for HSV-1 and HSV-2 were calculated by dividing the counts per minute obtained with the absorbed serum samples in tests against virus-infected cells by that obtained

against uninfected cells. A binding ratio of 2 or greater was considered indicative of the presence of antibody for the test HSV type. Patterns of binding ratios obtained with sera containing HSV-1, HSV-2, or both types of antibody are shown in Tables 1 through 5 (see below).

RESULTS

Optimal concentration of test sera for typing HSV antibodies. Preliminary studies were conducted to determine the appropriate dilutions of serum to use for typing HSV antibodies in the RIA system.

Figure 1 shows results of experiments in which serial dilutions of unabsorbed acute- and convalescent-phase sera from patients with initial HSV infections were tested for HSV antibody by the indirect RIA method. The patient with a type 1 infection had herpes stomatitis, and HSV-1 was isolated from a mouth swab. The type 2 patient had a genital infection, and HSV-2 was isolated from a lesion specimen.

Acute-phase sera, with the exception of the 1:1,000 dilution of serum from the type 2 infection, showed no significant reactivity with HSV-infected cells. Convalescent-phase sera showed high binding ratios for both HSV types at dilutions through 1:16,000 or 1:32,000.

Figure 2 shows antibody titers of convalescent-phase sera from the same HSV-1 and HSV-2 patients shown in Fig. 1 after absorption with uninfected, HSV-1-infected, and HSV-2-infected cells. In both cases, absorption of the serum with the infecting virus type removed reactivity against both serotypes. However, absorption with heterotypic virus removed common antibody, leaving specific antibody for the infecting virus type. The highest binding ratios (type-specific antibody activity) were demonstrable in the absorbed sera at dilutions from 1:1,000 to 1:8,000, and serum dilutions of 1:1,000 or 1:2,000 were employed for routine testing. Additional experiments indicated that these dilutions were also appropriate for detecting

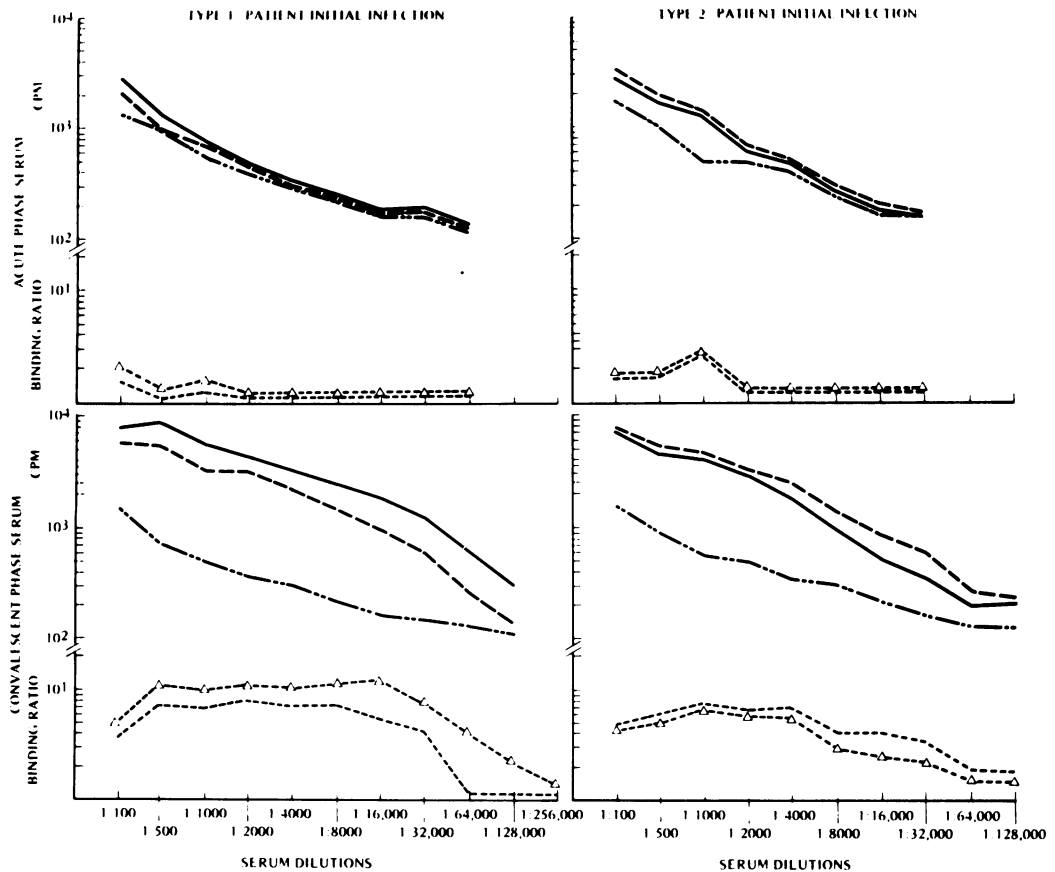


FIG. 1. HSV antibody titers determined by RIA on unabsorbed sera from patients with initial HSV-1 and HSV-2 infections. Symbols: —, tested versus HSV-1-infected cells; ---, tested versus HSV-2-infected cells; - · - · -, tested versus uninfected cells; Δ — Δ , binding ratio for HSV-1; — \bullet —, binding ratio for HSV-2.

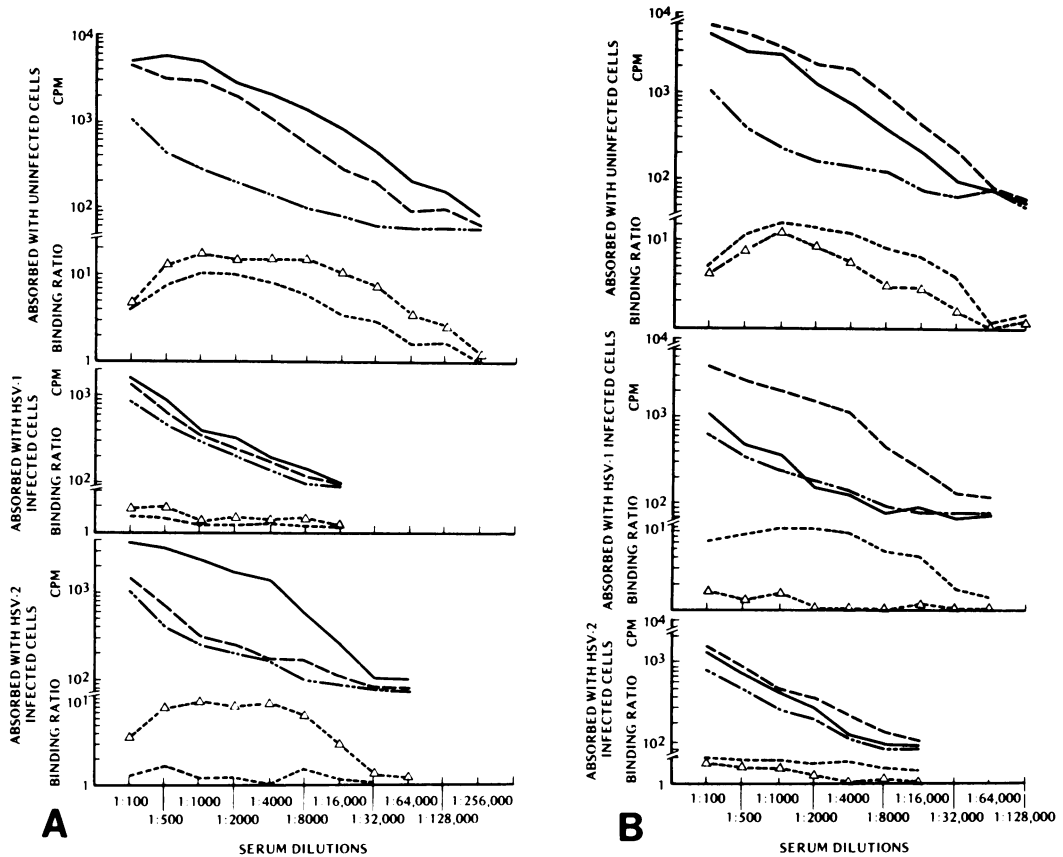


FIG. 2. HSV antibody titers determined by RIA on convalescent-phase sera after absorption with uninfected and infected cells. (A) HSV-1 infection; (B) HSV-2 infection. Symbols are as described in the legend to Fig. 1.

type-specific HSV antibody in sera from latent and recurrent HSV infections.

CSF showed little or no nonspecific activity in the RIA test system, and they could be examined for type-specific HSV antibody at dilutions of 1:100 or lower.

Optimal concentration of cells to use for absorption of sera. Studies were done to determine the optimal volume of packed cells to use for absorption of test sera. Sera from patients with an HSV-1 infection or an HSV-2 infection diluted 1:1,000 were absorbed with varying volumes of packed uninfected and infected cells to give ratios of packed cells to undiluted serum ranging from 2.5 to 20 (Fig. 3). At least 10 volumes of infected cells to 1 volume of undiluted serum was required for effective absorption of cross-reacting HSV antibodies. The use of larger volumes of cells for absorption occasionally resulted in slightly lower binding ratios for type-specific antibody, and therefore 10 volumes of cells was used routinely.

Examples of results obtained in typing HSV antibody in human sera and CSF. Examples of counts per minute and binding ratios obtained in typing HSV antibodies by the solid phase RIA method are shown in Tables 1 through 4. Table 1 shows results of antibody typing in an initial HSV infection in which HSV-1 was isolated from a mouth lesion. The absorbed acute-phase serum had no demonstrable antibody for either virus type, but the convalescent-phase serum absorbed with HSV-2 showed a significant binding ratio for HSV-1. Table 2 shows the results of antibody typing in an initial HSV infection in which HSV-2 was recovered from a genital lesion. The acute-phase serum had no demonstrable HSV antibody, but the convalescent-phase serum absorbed with HSV-1 showed residual, type-specific antibody for HSV-2. Table 3 shows results of antibody typing on a serum from an individual latently infected with both HSV types; HSV-1 was isolated at autopsy from the trigem-

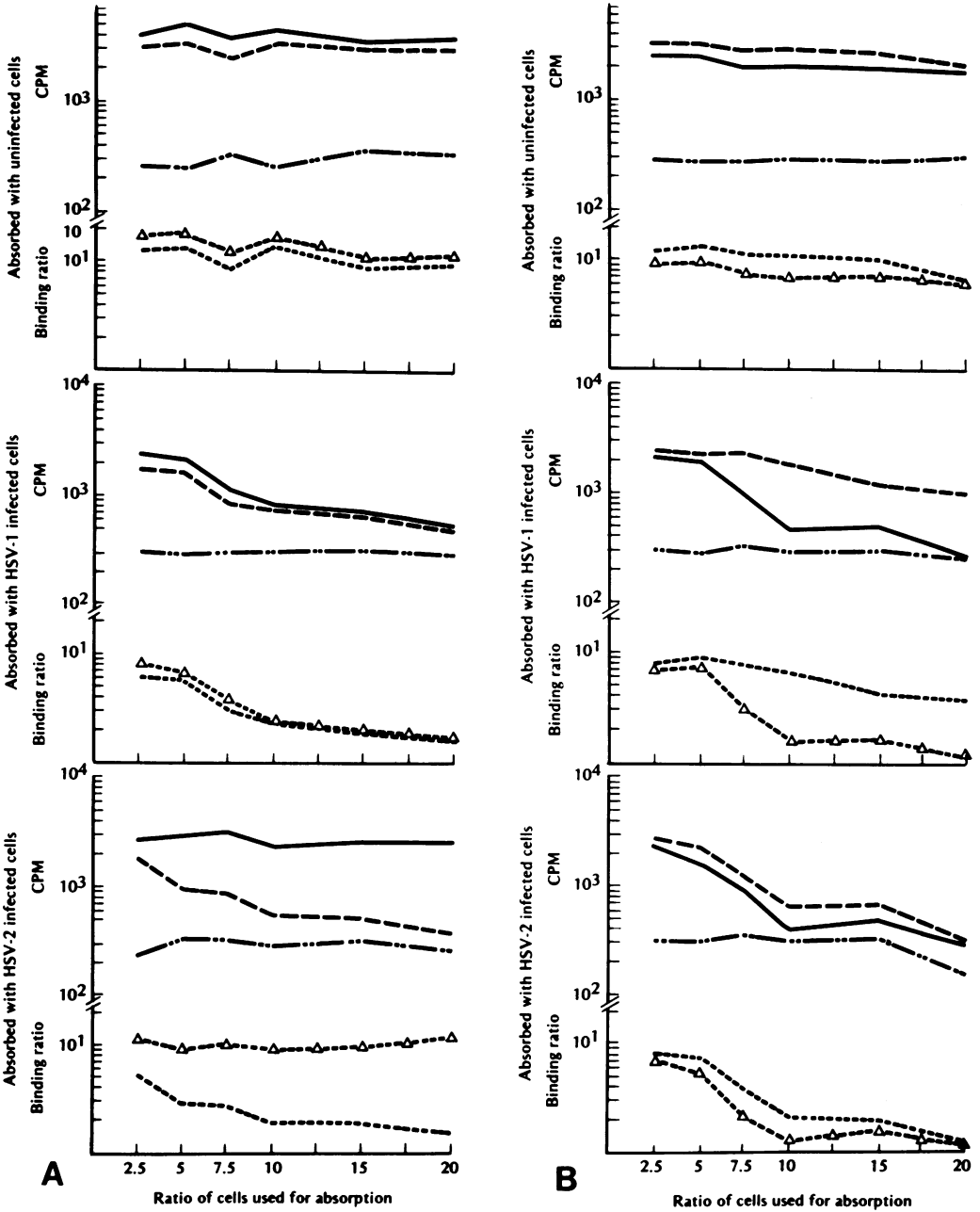


FIG. 3. Ability of various concentrations of HSV-infected cells to absorb common antibodies. (A) Serum from HSV-1 infection; (B) serum from HSV-2 infection. Symbols: —, HSV-1-infected cells; ---, HSV-2-infected cells; - · - ·, uninfected cells; Δ --- Δ , ratio to HSV-1; ----, ratio to HSV-2.

inal ganglion, and HSV-2 was isolated from the sacral ganglion (3, 4). The serum had a significant binding ratio for HSV-1 after absorption with HSV-2, and for HSV-2 after absorption with HSV-1, indicating the presence of antibody to both serotypes.

Table 4 shows results of typing HSV antibody in CSF by RIA. Even at a 1:100 dilution, the CSF gave less nonspecific binding to uninfected cells than is generally obtained with more dilute serum specimens, and this resulted in a very high specific binding ratio for HSV-1.

TABLE 1. *Typing of HSV antibodies by solid-phase RIA in a patient with an initial HSV-1 infection*

Patient's serum ^a tested against:	Counts/min					
	Acute-phase serum ^b absorbed with:			Convalescent-phase serum ^c absorbed with:		
	Uninfected HFDL cells	HSV-1- infected HFDL cells	HSV-2- infected HFDL cells	Uninfected HFDL cells	HSV-1- infected HFDL cells	HSV-2- infected HFDL cells
Uninfected HFDL cells	248	258	369	295	320	369
HSV-1-infected HFDL cells	254	242	357	2,924	508	2,087
HSV-2-infected HFDL cells	232	209	285	2,107	425	467
Binding ratios						
HSV-1	1.0	<1.0	<1.0	9.9	1.5	5.6
HSV-2	<1.0	<1.0	<1.0	7.1	1.3	1.2

^a Tested at a 1:2,000 dilution.^b Collected 4 days after onset of symptoms.^c Collected 31 days after onset of symptoms.TABLE 2. *Typing of HSV antibodies by solid-phase RIA in a patient with an initial HSV-2 infection*

Patient's serum ^a tested against:	Counts/min					
	Acute-phase serum ^b absorbed with:			Convalescent-phase serum ^c absorbed with:		
	Uninfected HFDL cells	HSV-1- infected HFDL cells	HSV-2- infected HFDL cells	Uninfected HFDL cells	HSV-1- infected HFDL cells	HSV-2- infected HFDL cells
Uninfected HFDL cells	350	273	289	329	291	342
HSV-1-infected HFDL cells	412	239	269	1,885	353	420
HSV-2-infected HFDL cells	369	275	303	2,425	1,563	395
Binding ratios						
HSV-1	1.1	<1.0	<1.0	5.7	1.3	1.2
HSV-2	1.0	1.0	1.0	7.3	5.3	1.1

^a Tested at a 1:2,000 dilution.^b Collected 5 days after onset of symptoms.^c Collected 19 days after onset of symptoms.TABLE 3. *Typing of HSV antibodies by solid phase RIA in an individual latently infected with HSV-1 and HSV-2*

Patient's serum ^a tested against:	Counts/min in serum absorbed with:		
	Uninfected HFDL cells	HSV-1- infected HFDL cells	HSV-2- infected HFDL cells
Uninfected HFDL cells	319	306	330
HSV-1-infected HFDL cells	2,690	441	1,158
HSV-2-infected HFDL cells	2,966	1,339	470
Binding ratios			
HSV-1	8.4	1.4	3.5
HSV-2	9.2	4.3	1.4

^a Tested at a 1:1,000 dilution.

Evaluation of the RIA method for typing HSV antibody. The sensitivity and specificity of the RIA method for typing HSV antibody in human sera was evaluated on certain sera in which the antibody type had been determined by other methods and on sera from individuals from whom HSV had been isolated and typed.

Table 5 shows the results of antibody typing by RIA on a panel of sera provided by A. J. Nahmias, on which the antibody had been typed by the microneutralization method (13). Serum number G449 had such a high level of HSV antibody that it was necessary to absorb it at a dilution of 1:2,000 and test at a dilution of 1:4,000; the other sera in this panel were absorbed at dilutions of 1:1,000 and tested at dilutions of 1:1,000. Results obtained by the two antibody-typing methods were in complete

TABLE 4. *Typing of HSV antibodies by solid phase RIA in CSF from a patient with meningoencephalitis*

Patient's CSF tested ^a against:	Counts/min					
	Acute-phase CSF ^b absorbed with:			Convalescent-phase CSF ^c absorbed with:		
	Uninfected HFDL cells	HSV-1-infected HFDL cells	HSV-2-infected HFDL cells	Uninfected HFDL cells	HSV-1-infected HFDL cells	HSV-2-infected HFDL cells
Uninfected HFDL cells	68	62	61	171	169	133
HSV-1-infected HFDL cells	87	89	70	6,138	283	3,310
HSV-2-infected HFDL cells	83	79	85	4,749	257	232
Binding ratios						
HSV-1	1.2	1.4	1.1	35.8	1.6	25.2
HSV-2	1.2	1.2	1.3	27.7	1.5	1.7

^a Tested at a 1:100 dilution.^b Collected 2 days after onset of symptoms.^c Collected 13 days after onset of symptoms.TABLE 5. *Results of HSV antibody typing by RIA on sera for which the antibody type had been determined by the microneutralization test*

Serum	Antibody type by microneutralization test	Binding ratio for HSV-1 after absorption with:			Binding ratio for HSV-2 after absorption with:			Antibody type by RIA
		Uninfected cells	HSV-1-infected cells	HSV-2-infected cells	Uninfected cells	HSV-1-infected cells	HSV-2-infected cells	
G440	1 and 2	11.1	1.0	4.2	8.5	2.1	1.4	1 and 2
G444	1 and 2	4.4	<1.0	1.7	5.2	2.0	1.1	?1 and 2
G448	2	8.0	1.5	1.3	11.0	8.2	1.3	2
G449	2	9.4	1.2	1.3	11.9	9.1	1.6	2
G456	1 and 2	8.3	<1.0	2.1	8.0	2.0	<1.0	1 and 2
G459	1	7.2	1.0	3.6	5.2	<1.0	1.2	1
G539	1	10.9	1.1	5.8	6.2	1.1	1.1	1
G586	1	6.1	1.2	3.3	4.2	1.2	1.0	1
G773	1	5.5	<1.0	4.0	2.8	<1.0	1.0	1

agreement, with the exception of an equivocal result obtained by RIA for HSV-1 antibody in serum G444, which was shown to contain antibody to both HSV-1 and HSV-2 by microneutralization. Antibody typing was also done by RIA on four sera which had been shown by inhibition of the indirect hemagglutination reaction to contain antibodies to both HSV-1 and HSV-2, and RIA also indicated the presence of antibody to both virus types.

In Table 6 results of antibody typing by RIA are shown for sera on individuals from whom HSV was isolated from clinical specimens in this laboratory. In all instances antibody was demonstrable for the virus type recovered from the patient. In five patients with genital herpetic infections, from whom HSV-2 was isolated, antibody to HSV-1 elicited by a previous

infection was also demonstrable. Three of these five patients had chronic HSV-2 infections, and binding ratios for the two virus types were similar in acute- and convalescent-phase sera. However, two of the five patients had initial HSV-2 infections, and binding ratios to both virus types increased between the acute- and convalescent-phase serum specimens.

Typing of antibody by RIA permitted identification of the infecting HSV type in a number of patients without virus isolations for whom laboratory diagnoses of HSV infection was based upon the demonstration of a fourfold or greater increase in complement-fixing antibody titer to an HSV-1 antigen (Table 6). For example, antibody to HSV-2 only was demonstrated in three patients with meningitis, and these infections were shown to be initial ones by the

development of significant binding ratios for HSV-2 between acute- and convalescent-phase sera. Antibody to HSV-1 was demonstrable in the spinal fluids of four patients with meningoencephalitis on whom virus isolation attempts were negative.

Table 7 shows results of antibody typing by RIA on sera from individuals on whom attempts were made by Richard Baringer to isolate HSV-1 from trigeminal ganglia and HSV-2 from sacral ganglia (3, 4). With a single exception, all of the 22 sera from individuals with HSV isolations had antibody for the virus type isolated. Serum from one individual from whom both HSV-1 and HSV-2 were isolated had antibody demonstrable only for HSV-1. All except one individual on whom virus isolation attempts were negative had antibody demonstrable by RIA to one or both HSV serotypes.

DISCUSSION

The high sensitivity of the indirect RIA method for detecting HSV antibody permits examination of test sera at high dilutions, and this makes cross-absorption of common antibodies feasible, since relatively small volumes of virus-infected cells are required. The use of an HSV antibody typing method based upon cross-absorption gives a more definitive identification of both types of antibody in the same serum specimen than do methods based upon differences in neutralization indices against HSV-1 and HSV-2 (13, 15).

Despite the fact that sera are absorbed with three cell preparations and then assayed against three types of cell cultures, the test is less cumbersome than most HSV antibody typing procedures, since a single dilution of serum or CSF can be examined. Sera from current

TABLE 6. Results of HSV antibody typing by RIA on sera and CSF from patients on whom laboratory diagnoses of HSV infection were made by virus isolation or serological findings

Clinical syndrome	No. of cases	Virus isolated	Antibody type by RIA ^a		
			HSV-1 only	HSV-2 only	HSV-1 and HSV-2
Meningoencephalitis	1	HSV-1	1	0	0
Meningoencephalitis or meningitis	5	None ^b	1	3	1
Meningoencephalitis or meningitis	4	None ^b	4 ^c	0	0
Gingivostomatitis	2	HSV-1	2	0	0
Gingivostomatitis	2	None ^b	2	0	0
Dermatitis	1	HSV-1	1	0	0
Dermatitis	1	HSV-2	0	1	0
Dermatitis	2	None ^b	2	0	0
Genital tract infection	11	HSV-2	0	6	5
Genital tract infection	3	None ^b	0	2	1

^a Sera tested at a 1:2,000 dilution.

^b Serologic diagnosis made by $\geq 4\times$ rise in complement fixation antibody titer to HSV.

^c Antibody typed in CSF.

TABLE 7. Results of antibody typing by RIA on sera from individuals on whom attempts were made to isolate HSV from sensory nerve ganglia

Virus isolation results ^a	No. of individuals	No. with HSV antibody by RIA ^b to:			
		HSV-1 only	HSV-2 only	HSV-1 and HSV-2	Neither virus
HSV-1 pos., HSV-2 neg.	13	11	0	2	0
HSV-1 neg., HSV-2 pos.	2	0	2	0	0
HSV-1 pos., HSV-2 pos.	3	1	0	2	0
HSV-1 pos., HSV-2 NT	3	3	0	0	0
HSV-1 NT, HSV-2 pos.	1	0	0	1	0
HSV-1 neg., HSV-2 neg.	15	9	1	4	1

^a pos., Positive; neg, negative; NT, not tested.

^b Sera tested at a dilution of 1:1,000.

HSV infections are generally examined at a dilution of 1:2,000, and those from past infections are examined at a dilution of 1:1,000. Occasionally a serum contains such high levels of HSV antibody that absorption by the standard method does not effectively remove antibody; in these instances antibody typing can be made clear-cut by absorbing the serum at a 1:2,000 dilution and testing at a dilution of 1:4,000.

The number of virus-infected cells required to detect residual HSV antibody by RIA is small, approximating that used for cover slip cultures, and the fact that these cell cultures can be stored indefinitely after acetone fixation is also an advantage, as it permits prior preparation of these materials for use in large-scale studies.

In addition to its sensitivity for typing HSV antibody in human sera, the RIA method has been particularly useful for detecting and typing antibody in CSF, as it is sensitive enough to detect antibody in certain specimens which are negative, or anticomplementary, by complement fixation. The procedure is also useful for type-specific serodiagnosis of HSV infections in patients without virus isolations; it has shown HSV-2 to be the etiologic agent in a number of cases of meningitis in which virus isolation attempts were not done or were negative.

Our findings would indicate that a clear-cut, type-specific antibody response to HSV-2 does indeed occur in individuals who have experienced a prior infection with HSV-1. In either primary or chronic HSV-2 infections in individuals with pre-existing HSV-1 antibody, the RIA method clearly demonstrated antibody to HSV-2, and binding ratios were usually higher for HSV-2 than for HSV-1. Using a ^{51}Cr release test to detect cytolytic antibodies to HSV-2-infected cells, Smith et al. (17) found that many individuals with HSV-2 infections who had experienced a previous infection with HSV-1 produced little or no cytolytic antibody to HSV-2. On the other hand, HSV-2 patients without serological evidence of a prior HSV-1 infection produced significant levels of cytolytic antibody to HSV-2-infected cells. These investigators suggested that the antigenic relationship between the two HSV types might be similar to that of the influenza A viruses, and that subsequent infection with the HSV heterotype might serve largely to stimulate an antibody response to the initial infecting type, whereas the antibody response to the second virus is minimal. It appears more likely, however, that the ^{51}Cr -release test of Smith et al. (17) and our RIA method measure antibodies produced to different HSV antigenic determinants.

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