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Differentiation of Serum Antibodies to Herpesvirus Types 1 and 2 by Radioimmunoassay (40036)

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Herpes simplex virus types 1 (HSV-1) and 2 (HSV-2) share common antigens and possess type specific antigens (1). Much of the antibody directed against HSV-2 also reacts with antigens shared with HSV-1 after infection with either virus type. This makes serologic diagnosis of HSV-2 infections difficult because many adults have had HSV-1 infections before becoming infected with HSV-2 (2). We were able to detect antibody to HSV-1 and HSV-2 using a solid phase radioimmunoassay (RIA). By adsorption with miniature affinity columns of crushed polystyrene coated with HSV-1, much of the antibody directed against shared antigens could be removed, allowing detection of antibody to HSV-2 type specific antigens by RIA.

Materials and methods. Tissue culture. Cultures consisted of monkey kidney (VERO) cells grown in basal medium Eagles with 10% fetal calf serum (FCS), glutamine (2 m*M*/ml) and neomycin (20 μg/ml). Strains of HSV-1 (Patton) and HSV-2 (333) were kindly provided by Dr. F. Rapp. Cell monolayers in 32-ounce prescription bottles were infected with virus and incubated until >90% of all cells showed cytopathic effect (about 20 h). Infected cells were washed twice with phosphate buffered saline (PBS) and frozen at −70°.

Adsorbents. Adsorbents were prepared by crushing polystyrene test tubes (Falcon, No. 2025) into a fine powder with the aid of a Sorvall omnimixer. This powder was washed with PBS and approximately 40 ml wet powder placed into a 100 ml beaker. Antigen from three 32-ounce bottles of HSV-1 infected cells was thawed, sonicated and diluted with 20 ml PBS. This was mixed with a magnetic stirrer for 1 h at room temperature. The adsorbent was poured into a #200 sieve (Cistron Corp., Elmsford, N.Y.) and washed with 100 ml of 10% FCS. Control adsorbent was prepared with 10% FCS in place of HSV-1. Adsorbent (approximately 4 ml) was then put into small disposable columns (Quik-Step columns, Isolab, Inc., Akron, OH) and were ready for immediate use. In experiments where adsorption was utilized, sera were diluted 1:200 in 10% FCS in PBS and tumbled overnight at 4° either with HSV-1 coated polystyrene (referred to as adsorbed sera) or with FCS coated polystyrene (referred to as unadsorbed sera).

Serum samples. Forty-eight coded serum samples from women with different histories of herpetic infection were obtained for analysis. The first group of sera was collected from eighteen nuns who denied ever having sexual intercourse. A second group was collected from eighteen prostitutes, all of whom admitted to having multiple sex partners. The third group of sera was collected from ten asymptomatic women from whom HSV-2 was isolated and identified during blind screening (3). Also included in these forty-eight sera were one serum from an individual negative for HSV-1 and HSV-2 and sera from two individuals infected with HSV-2 confirmed by virus isolation from genital lesions. It was reasoned that the nuns would be low risk for HSV-2 infections and the prostitutes would be at high risk for HSV-2 infections, although virus isolation data was not available on these two groups. Two uncoded sera were selected for preliminary experiments from two persons who had previous infections with HSV-1 only or HSV-2 only, confirmed by virus isolation.

RIA Procedures. All sera were diluted 1:200 in 10% FCS in PBS and tested with HSV-1, HSV-2, and VERO antigens as previously described (4). Briefly, plastic-coated beads were coated with antigen and reacted overnight with patients' sera. Excess serum was washed off and beads were reacted 4 h with ^{125}I-labeled antiglobulin, washed and the radioactivity on the beads

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counted in a α-counter. All washings were carried out with a specially designed magnetic transfer device capable of performing multiple assays simultaneously (5). The HSV-1 antigen used to coat beads was diluted to give a reaction in cpm approximately equal to the reaction with HSV-2 antigen when tested with pooled serum from patients infected with HSV-1 followed by HSV-2.

^{51}Cr release test. Antibodies to HSV-1 and HSV-2 infected cells were quantitated using the release of ^{51}Cr in the presence of guinea pig complement (6). To detect type specific antibodies to virus infected cells, the sera were adsorbed with cells infected with the heterologous virus type (6).

Results. In order to determine the effect of serum adsorption with HSV-1 coated polystyrene, two sera were tested. The first serum was obtained from a person infected with HSV-1 only. Before adsorption this serum gave a reaction of about 2000 cpm higher with HSV-1 antigen than with HSV-2 antigen (Fig. 1). Essentially all antibody to HSV-1 type specific and common antigens were removed by adsorption as indicated by no reaction with HSV-1 or HSV-2 antigens following adsorption. A second serum from an individual infected with HSV-2 only was also tested. This serum reacted approximately 4000 cpm higher with HSV-2 antigen than with HSV-1 antigen before adsorption, an opposite pattern than that

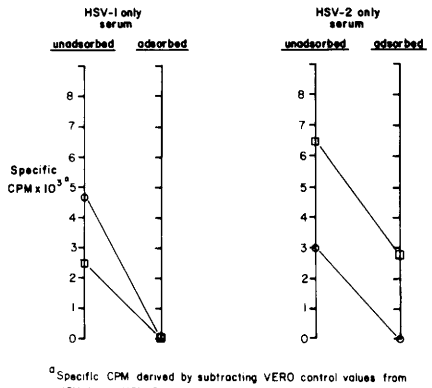

FIG. 1. Effect of serum adsorption with HSV-1 coated polystyrene. Specific cpm were derived by subtracting VERO control values from HSV-1 and HSV-2 values, O——O = HSV-1, □——□ = HSV-2.

observed with the serum from the individual infected with HSV-1 only. Following adsorption, essentially all antibody to the common antigen of HSV-1 was removed as indicated by lack of detectable reaction with HSV-1 antigen. Antibody to HSV-2 antigen was then clearly detectable at approximately 2700 cpm above background.

Serum samples from forty-eight women were then tested under code for antibody to HSV-1, HSV-2 and uninfected VERO control cell antigens. These sera were initially tested without adsorption by RIA. Serum reactivities in cpm with each antigen were determined and ratios were calculated by dividing cpm obtained with HSV-2 antigen by cpm obtained with HSV-1 antigen (Table I). The HSV-2/HSV-1 ratios ranged from -1.0 to $+4.6$, those sera with lower ratios giving higher reactions with HSV-1 than with HSV-2 antigen. As the HSV-2/HSV-1 ratio becomes larger, serum reactivity with HSV-2 antigen increases relative to the reaction with HSV-1 antigen.

A plot of this RIA data indicates at least two populations within the distribution of HSV-2/HSV-1 ratios (Fig. 2). Those sera with low ratios indicate a preponderance of antibody to HSV-1 antigen and those with higher ratios indicate more antibody to HSV-2 antigen. An intermediate group of sera between the two populations was tested by serum adsorption in an attempt to remove antibody to common antigens and identify those women with antibody to HSV-2 type specific antigens.

The data in Table II show the HSV-2/HSV-1 ratios before and after adsorption and the origin of the serum samples after the code was broken. If a serum gave a ratio of a greater than or equal to 1.0, it was considered positive for antibody to HSV-2 type specific antigen. The reason for using this criterion is that the HSV-1 and HSV-2 antigens used in this RIA were diluted to give approximately equal reactions when tested with pooled sera from individuals infected with HSV-1 followed by HSV-2. Six of the sera tested by adsorption converted to ratios of greater than 1.0 after adsorption; demonstrating the effect of adsorption, which was to remove antibody directed against the shared antigens

TABLE I. RADIOIMMUNOASSAY OF CODED SERUM SAMPLES

Number	cpm × 10^3 HSV-1 antigen	HSV-2 antigen	Cell control antigen	Ratio[a] HSV-2/HSV-1
1	1.0	0.4	0.7	−1.0
41	1.0	0.6	0.4	0.3
28	2.7	1.2	0.4	0.4
30	2.4	1.1	0.5	0.4
23	4.0	1.9	0.5	0.4
26	1.9	1.1	0.5	0.4
17	4.6	2.4	0.7	0.4
14	3.0	1.7	0.6	0.5
16	3.2	1.8	0.6	0.5
27	3.5	1.9	0.5	0.5
20	4.1	2.2	0.5	0.5
24	2.8	1.7	0.6	0.5
13	4.3	2.4	0.5	0.5
5	5.2	3.1	0.7	0.5
45	3.6	2.2	0.6	0.5
39	4.1	2.3	0.6	0.6
15	4.2	2.6	0.6	0.6
29	4.4	2.8	0.6	0.6
21	3.7	2.4	0.6	0.6
7	5.6	3.6	0.5	0.6
34	5.0	3.4	0.9	0.6
3	4.9	3.3	0.7	0.6
19	2.5	1.7	0.4	0.6
25	5.2	3.5	0.7	0.6
12	3.7	2.5	0.4	0.6
47	4.5	3.1	0.6	0.6
44	3.5	2.3	0.5	0.6
18	3.0	2.2	0.4	0.7
42	4.2	3.1	0.6	0.7
38	6.2	4.6	0.9	0.7
46	3.3	2.7	1.0	0.7
33	5.6	4.6	1.1	0.8
40	2.8	2.3	0.5	0.8
11	6.2	5.1	0.5	0.8
9	5.6	4.6	0.3	0.8
43	4.3	3.7	0.6	0.8
8	3.5	3.0	0.4	0.8
22	4.9	4.3	0.7	0.9
6	2.8	2.5	0.5	0.9
10	7.4	6.7	0.3	0.9
31	4.7	4.8	0.8	1.0
32	2.7	2.9	1.0	1.1
36	5.1	7.3	0.5	1.5
48	1.6	2.1	0.8	1.6
35	1.5	2.3	0.7	2.0
37	2.8	4.6	1.0	2.0
2	2.8	5.3	0.6	2.1
4	1.4	3.9	0.7	4.6

[a] Means HSV-2 counts minus VERO counts divided by HSV-1 counts minus cell control counts.

so that HSV-2 type specific antibody could be detected.

After breaking the serum code it was revealed that serum numbers 4–12 came from asymptomatic women from whom HSV-2 was isolated during blind screening. The second group of sera, numbers 13–30, came from nuns and the third group of sera, numbers 31–48, came from prostitutes. Also included were one seronegative control (serum number 1) and two seropositive controls (sera numbers 2 and 3) for HSV-2 confirmed by virus isolation and serum neutralization. It was reasoned that the nuns would be at low risk for HSV-2 infections and the prostitutes would be at high risk for HSV-2 infections, although virus isolation data were not available on these two groups.

The results summarized in Table III indicate which serum samples were positive for HSV-2 antibody measured by RIA and ^{51}Cr release methods. All sera (except #32) were titered, then diluted to contain 5 units of antibody, adsorbed with HSV-1 infected cells and then assayed in the ^{51}Cr release test against HSV-2 infected cells. Greater than 2% specific ^{51}Cr release is considered significant (6) for the presence of HSV-2 antibody. It can be concluded that all the nuns' sera were negative for HSV-2 antibody by both assays, meaning that there were no false positive. Ten of eighteen prostitutes' sera were positive by RIA compared with three positives out of seventeen prostitutes' sera tested by ^{51}Cr release. Of the nine asymptomatic women, three were positive by RIA and two were positive by ^{51}Cr release. This group of asymptomatic women would contain those individuals likely to make little if any detectable antibody response to an inapparent HSV-2 infection for reasons in the discussion.

A comparison of RIA and ^{51}Cr release assay was also made by plotting HSV-2/HSV-1 RIA ratios versus II/I cytotoxic antibody indices obtained by ^{51}Cr release assay (Fig. 3). The nuns' sera gave lower ratios and indices than did the prostitutes and asymptomatic women. A partial correlation between the two methods appears to exist except for four prostitutes' sera and one serum sample from an asymptomatic woman, all of which had high HSV-2/HSV-1 RIA ratios.

Discussion. It has been reported that persons infected with HSV-1 or HSV-2 alone

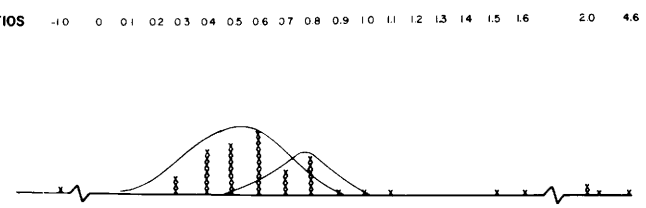

FIG. 2. RIA ratios, HSV-2/HSV-1, on coded serum samples. Each X represents a serum sample. RIA ratios were calculated according to the formula: HSV-2 minus VERO ÷ HSV-1 minus VERO. Values (in cpm) were determined by RIA with each of the forty-eight sera using HSV-1, HSV-2 and VERO antigens.

TABLE II. EFFECT OF SERUM ADSORPTION WITH HSV-1 COATED POLYSTYRENE. RATIO MEANS HSV-2 CPM DIVIDED BY HSV-1 CPM.

Unadsorbed			Absorbed		
Serum No.	Group	Ratio	Serum No.	Group	Ratio
47	prostitute	0.6	10	asymptomatic	0.6
44	prostitute	0.7	44	prostitute	0.6
18	nun	0.7	6	asymptomatic	0.7
42	prostitute	0.7	22	nun	0.8
38	prostitute	0.7	42	prostitute	0.8
46	prostitute	0.7	18	nun	0.8
33	prostitute	0.8	47	prostitute	0.8
40	prostitute	0.8	9	asymptomatic	0.8
11	asymptomatic	0.8	33	prostitute	0.9
9	asymptomatic	0.8	43	prostitute	1.1
43	prostitute	0.8	8	asymptomatic	1.1
8	asymptomatic	0.8	46	prostitute	1.1
22	nun	0.9	40	prostitute	1.2
6	asymptomatic	0.9	11	asymptomatic	1.2
10	asymptomatic	0.9	32	prostitute	1.3
31	prostitute	1.0	38	prostitute	1.5
32	prostitute	1.1	31	prostitute	2.0

produce a population of antibody of which approximately 80% is directed against the cross-reacting or common antigen shared by the two viruses. The remainder of the antibody (20%) is produced against type specific antigens (6). Most humans become exposed to HSV-1 before reaching sexual maturity, at which time a relatively smaller percent of the population acquire infection with HSV-2. If a person has antibody to HSV-1 and becomes infected with HSV-2, the antibody production shifts to 90% against shared antigen indicating an anamestic response, 5–10% against HSV-1 specific antigens and a minimal response to HSV-2 specific antigens as measured by complement dependent lysis of infected cells. A possible explanation for those asymptomatic women positive by HSV-2 virus isolation and negative by RIA could be that high levels of preexisting serum neutralizing antibody to the common antigen obtained from an earlier infection with HSV-1 prohibits these women from responding to the HSV-2 type specific antigens upon infection with this virus.

In conclusion, the advantages of this RIA for serological detection of past HSV-2 infection include its simplicity and applicability to testing a large number of serum samples rapidly at low cost without the use of purified antigen. By using HSV-1 coated polystyrene adsorbents in miniature affinity columns, antibody to the shared antigens of HSV-1 and HSV-2 could be removed. These procedures enhance detection of antibody to HSV-2 type specific antigens, allowing a better assessment of the humoral response to infection with HSV-1 and HSV-2. Finally, this technique is an economic

TABLE III. HSV-2 ANTIBODY IN CODED SERUM SAMPLES.

		HSV-2 Antibody	
Serum No.	Source	Ria Positive/Total	^{51}Cr Release[a] Positive/Total
4–12	Asymptomatics with HSV-2 Isolation	3/9	2/9
13–30	Nuns	0/18	0/18
31–48	Prostitutes	10/18	3/17

[a] Sera diluted to contain 5 units of antibody, adsorbed with HSV-1 infected cells and then tested in the ^{51}Cr release assay (6).

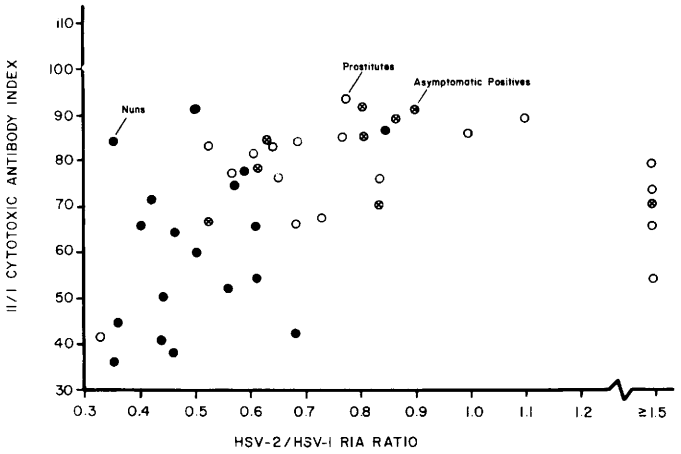

FIG. 3. Comparison of RIA and ^{51}Cr release assay. HSV-2/HSV-1 RIA ratios were determined by dividing serum reactivity in cpm with HSV-2 antigen by serum reactivity with HSV-1 antigen. II/I cytotoxic antibody indices were calculated accordingly = II/I index = titer (HSV-2) $\log_{10}$/titer (HSV-1) $\log_{10} \times 100$. Titers were determined by ^{51}Cr release (6).

alternative to the use of a highly purified type specific antigen recently reported by Knez *et al.* (7).

Summary. Antibody against herpes virus types 1 (HSV-1) and 2 (HSV-2) was detected in a coded test of forty-eight coded human sera by radioimmunoassay (RIA). Antibody to antigens shared between the two virus types could be removed by adsorption with HSV-1 coated polystyrene allowing detection of HSV-2 specific antibody. No false positives were observed in eighteen women at low risk for HSV-2 infection. HSV-2 specific antibody was detected in ten of eighteen women who were at high risk for HSV-2 infection. Antibody to HSV-2 was detected in three of ten asymptomatic women. Biological variation in response to HSV-2 could provide an explanation for those asymptomatic women who were positive by virus isolation, but negative for HSV-2 type specific antibody by RIA.

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