

Sensitivity of a Radioimmunoassay Method for Detection of Certain Viral Antibodies in Sera and Cerebrospinal Fluids

BAGHER FORGHANI, NATHALIE J. SCHMIDT, AND EDWIN H. LENNETTE*

Viral and Rickettsial Disease Laboratory, California State Department of Health, Berkeley, California 94704

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An indirect solid-phase radioimmunoassay (RIA) was applied to titration of serum and cerebrospinal fluid (CSF) antibodies against a variety of viruses including rubella, mumps, measles, herpes simplex, varicella-zoster, and vaccinia. The test used fixed, virus-infected cells as a source of antigen, and conditions for optimal production of viral antigen were determined for each virus-host cell system. In acute, uncomplicated viral infections, sera taken 2 to 5 days after onset generally had low homotypic RIA titers ranging from <1:100 to 1:500, whereas convalescent-phase titers ranged from 1:128,000 to 1:512,000. Rubella and measles antibody titers as high as 1:256,000 were demonstrated by RIA in CSF from patients with chronic panencephalitis, whereas homologous antibody titers of 1:4,000 were detected in CSF from acute mumps, herpes simplex, and varicella-zoster virus infections with central nervous system involvement. Some heterotypic antibody was demonstrable by RIA in CSF, but, with the exception of herpes simplex antibody in a mumps virus infection, titers were markedly lower than those to the infecting virus type. RIA generally demonstrated titers at least 1,000 times higher than those obtained by conventional assays such as complement fixation, hemagglutination inhibition, neutralization, and immunofluorescent staining.

Radioimmunoassay (RIA) combines the high sensitivity of radioisotope labeling with the marked specificity of immunological reactions, and, since the development of an RIA method by Yalow and Berson in 1960 (26) for determination of insulin in plasma, the procedure has been widely applied in the field of clinical chemistry for assay of trace amounts of a variety of biological substances.

With the exception of assays for hepatitis B surface antigen and antibody (13, 17), RIA has not had wide use in diagnostic virology. Hepatitis B surface antigen has unique features that contribute to the sensitivity and reliability of RIA. These include its high degree of antigenic stability and the fact that it is present in high concentration in serum, unassociated with host tissue, and thus can be readily isolated, purified, and concentrated. Since other viral antigens do not possess these characteristics, development of sensitive RIA techniques has been more difficult. Some of the RIA methods described for assay of viral antibodies have had little or no greater sensitivity, from the standpoint of antibody titers demonstrated, than conventional viral antibody assay methods such as complement fixation (CF), hemagglutination inhibition (HI), neutralization, or fluorescent-

antibody (FA) staining (10, 12, 27), and thus there has been no particular advantage to the diagnostic laboratory in adopting these methods, which require unstable, and potentially hazardous, radioisotopes and special equipment for counting isotope emissions.

RIA procedures with greater sensitivity than standard methods have been developed in this laboratory for the type-specific identification of herpes simplex virus (HSV) (4) and antibody (5). This report describes the extension of indirect solid-phase RIA to detection of antibodies to certain other important human viruses in sera and cerebrospinal fluids (CSF) and compares the sensitivity of RIA to that of conventional viral antibody assays.

MATERIALS AND METHODS

Virus-infected cells. Virus-infected cell cultures in the bottom of 1-dram glass vials were used as a source of antigen for assay of antibodies by indirect RIA. Uninfected cells of the same type prepared in parallel were used as controls. Growth medium for the cell cultures was 90% Eagle minimum essential medium with 10% fetal bovine serum, and maintenance medium was 98% Eagle minimum essential medium with 2% fetal bovine serum.

Rubella virus-infected cell cultures were prepared using the RV virus strain (originally obtained from

J. L. Sever, National Institutes of Health) and the BHK-21 line of baby hamster kidney cells. Cells in suspension were inoculated at a ratio of 1 plaque-forming unit (PFU) of virus per cell and incubated at 37°C for 1 h, and 90,000 to 100,000 cells in 1 ml of growth medium were planted in 1-dram vials. After incubation at 36°C in a CO₂ incubator for 24 h, the growth medium was replaced with 1 ml of maintenance medium, and cultures were incubated for an additional 3 days. The medium was then removed, the vials were rinsed with 1 ml of distilled water, and, without drying, the cells were fixed with acetone for 20 min and then stored at -70°C for use as required.

Mumps virus-infected cell cultures were prepared using the Barnes virus strain, an isolate from this laboratory, and the Vero line of African green monkey kidney cells. Monolayer cultures were produced by seeding vials with 50,000 cells in 1 ml of growth medium and incubating for 1 to 2 days at 36°C. Cultures in 1 ml of maintenance medium were then infected at a ratio of approximately 1 PFU of virus per cell and incubated at 36°C for 3 to 4 days, at which time they showed a 2 to 3-plus viral cytopathic effect (CPE). Cells were fixed and stored as described above.

The LEC strain of measles virus, isolated from the brain of a child with subacute sclerosing panencephalitis (1), was obtained from H. Koprowski, The Wistar Institute, Philadelphia, Pa. It was propagated in human fetal diploid lung (HF₂DL) cell cultures, which were initiated by seeding vials with 50,000 to 60,000 cells in 1 ml of growth medium. After incubation at 36°C for 2 to 3 days, the growth medium was replaced with 1 ml of maintenance medium and cultures were infected at a ratio of approximately 1 PFU of virus to 5 cells. After 4 to 5 days of incubation at 36°C, the cultures showed the syncytial formation characteristic of LEC virus CPE, and they were fixed and stored at -70°C.

The MacIntyre strain of HSV type 1, isolated in this laboratory from a fatal case of encephalitis, was used for preparation of virus-infected HF₂DL cell cultures; complete details are given elsewhere (4, 5).

Varicella-zoster (V-Z) virus-infected cell cultures were prepared using the Batson virus strain, isolated in this laboratory from a zoster lesion, and HF₂DL cells. Monolayer HF₂DL cultures prepared as described for measles virus were infected with cell-free virus (20) at a ratio of approximately 1 PFU per cell. After 2 to 3 days of incubation at 36°C, when the cultures showed a 3-plus CPE, they were acetone fixed and stored at -70°C.

The IHD strain of vaccinia virus, obtained from the American Type Culture Collection, was propagated in HF₂DL monolayers prepared as described for measles virus. Cultures in 1 ml of maintenance medium were infected at a ratio of approximately 1 PFU of virus to 5 cells. After 18 h of incubation the cultures showed a 2- to 3-plus viral CPE, and they were acetone fixed and stored at -70°C.

Immunofluorescent staining indicated that viral antigen was present in 90 to 100% of the cells in infected cultures at the time they were harvested for use in RIA.

RIA. The purification and radiolabeling of anti-human globulins and the solid-phase RIA procedure for antibody assay have been described in detail (4, 5). Briefly, antiserum produced in goats against human gamma globulin was obtained from Antibodies Inc., Davis, Calif. The gamma globulin was precipitated by one-third saturation with ammonium sulfate and further purified by diethylaminoethyl-cellulose chromatography. The purified immunoglobulin G (IgG) was labeled with ¹²⁵I by the chloramine T method (9, 19), and the specific radioactivity was approximately 0.5 μ Ci per μ g of protein. For antibody assays, dilutions of serum or CSF prepared in 0.01 M phosphate-buffered saline, pH 7.2 (PBS), were reacted with virus-infected and uninfected cells overnight at room temperature. After removal of the specimen and washing with PBS, the presence of antibody complexed with viral antigen in the cells was detected by the addition of the ¹²⁵I-labeled anti-human gamma globulin. After incubation at room temperature for 70 to 80 min, followed by thorough rinsing of the vials with PBS, residual radioactivity was assayed directly in the vials with a gamma counter (Beckman Gamma 300 radiation counter). Counts per minute in duplicate tests were averaged and binding ratios were calculated by dividing the counts per minute obtained with the specimen against virus-infected cells by the counts per minute obtained against uninfected cells. Binding ratios of 2 or greater were considered indicative of the presence of specific viral antibody.

Conventional viral antibody assays. CF (14), HI (2, 8), neutralization (19) and FA (15, 16, 21) tests were performed by the standard procedures used in this laboratory, which have been described in detail.

Sera and CSF. All sera and CSF examined for antibody by RIA were from the diagnostic files of this laboratory and were from patients in whom a viral infection had been diagnosed by virus isolation and/or conventional serological assays. Further details on representative patients are given below.

RESULTS

Titers of homologous antibody detected by RIA in sera from acute viral infections. Figures 1 through 5 show the counts per minute and binding ratios obtained in RIA titrations of homologous viral antibody in representative patients with acute, uncomplicated viral infections.

Figure 1 shows the results of RIA titrations of rubella antibody in acute-phase (2 days after onset) and convalescent-phase (25 days after onset) sera from a representative postnatal rubella virus infection that occurred in a 29-year-old male. Significant antibody activity was demonstrable only through the 1:500 dilution of acute-phase serum, but was detectable through the 1:256,000 dilution of convalescent serum.

Figure 2 depicts RIA titrations of mumps virus antibody in acute-phase (3 day) and convalescent-phase (26 day) sera from an infection

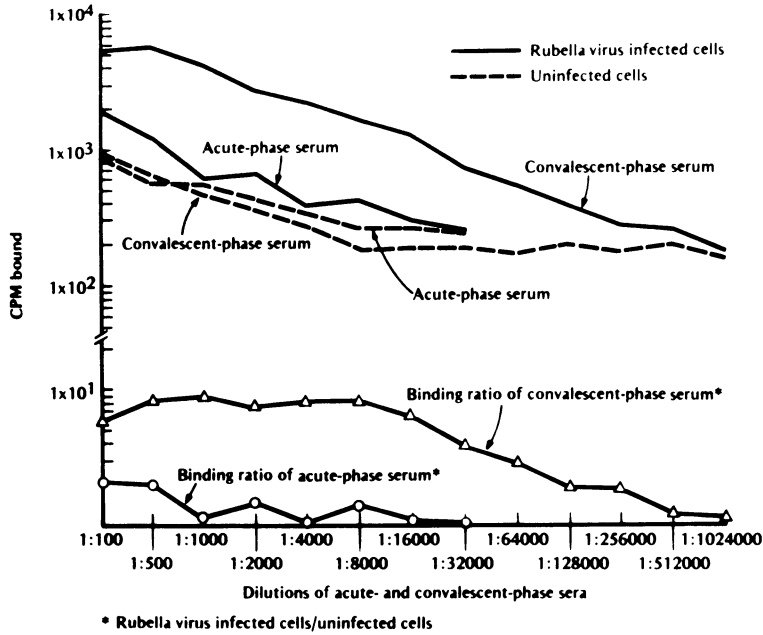


FIG. 1. Titration of rubella virus antibody in acute- and convalescent-phase sera by indirect RIA.

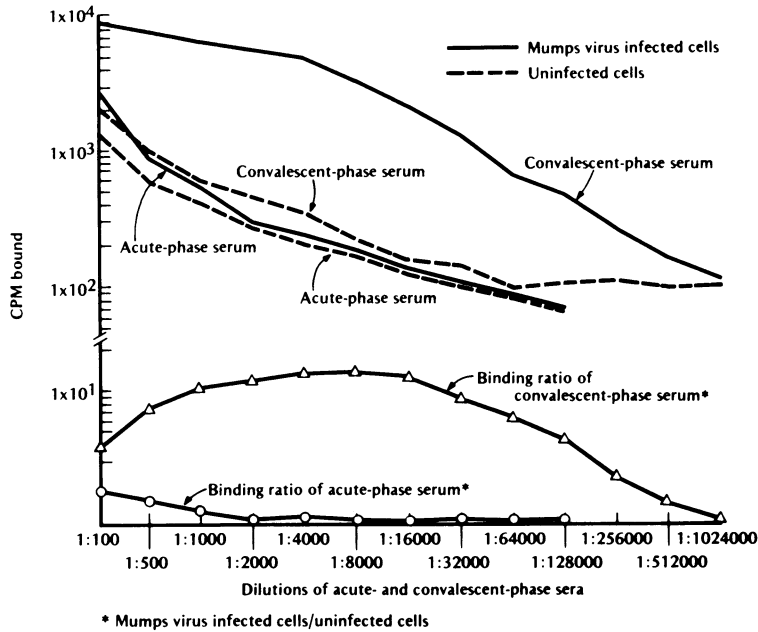


FIG. 2. Titration of mumps virus antibody in acute- and convalescent-phase sera by indirect RIA.

in a 14-year-old female in whom parotitis was the major clinical finding. The infection was initially diagnosed by demonstration of a significant antibody titer rise to mumps CF antigen. The convalescent-phase serum showed an

tibody activity through the 1:256,000 dilution.

In Fig. 3 it is seen that convalescent-phase serum (22 day) from an uncomplicated measles virus infection in a 14-year-old male showed homologous antibody activity by RIA at dilu-

tions through 1:512,000, whereas the acute-phase serum (1 day) had a titer of only 1:500.

Figure 4 shows the results of RIA titrations

for V-Z virus antibody in acute-phase (5 day) and convalescent-phase (20 day) sera from a varicella infection in an 18-year-old male. The

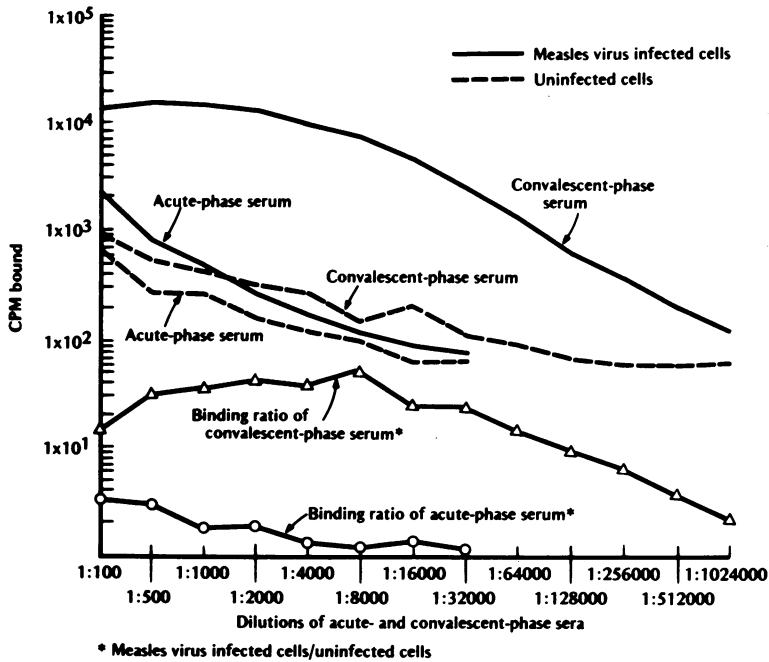


FIG. 3. Titration of measles virus antibody in acute- and convalescent-phase sera by indirect RIA.

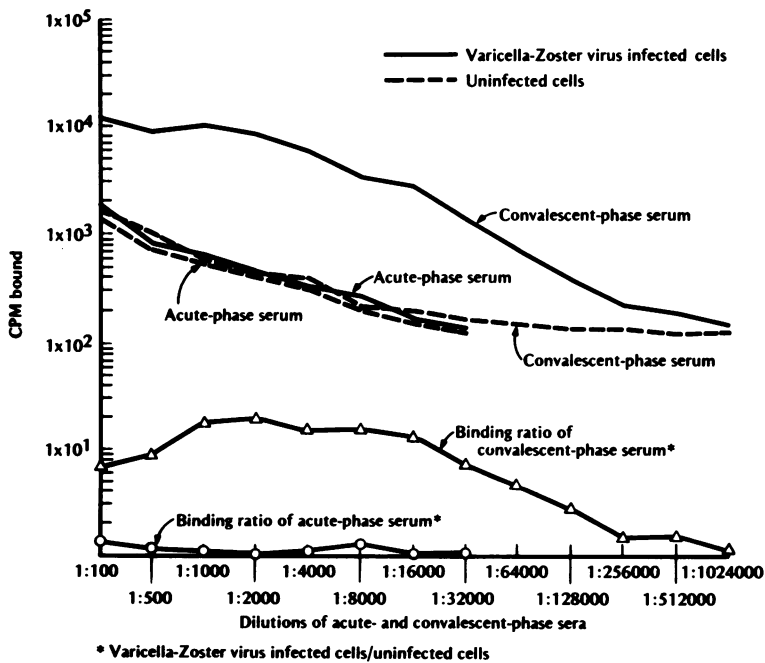


FIG. 4. Titration of varicella-zoster virus antibody in acute- and convalescent-phase sera by indirect RIA.

convalescent-phase serum had a titer of 1:128,000, and the acute-phase serum had a titer of <1:100.

In Fig. 5 results are shown of an RIA titration for vaccinia antibody in a serum from a 63-year-old female in whom vaccinia infection was diagnosed by FA staining of a vesicular lesion. As a negative control, a serum from an unvaccinated infant was tested in parallel. The serum from the infection showed specific antibody at dilutions through 1:128,000, and the infant's serum had no demonstrable antibody activity.

In Fig. 1 through 5, the titration curves of convalescent-phase sera against the various viruses were similar in that lower dilutions of the sera reacted more strongly with uninfected cells and thus gave lower specific binding ratios; with increased dilution of the sera, reactivity with uninfected cells tended to plateau, and specific binding ratios were higher.

Titration of viral antibodies in CSF by RIA. Figures 6 through 10 show the results of RIA titrations of viral antibody in CSF of representative patients with central nervous system (CNS) manifestations of viral infection.

In Fig. 6 results are shown of an RIA titration of rubella virus antibody in the CSF of an 11-year-old male with chronic progressive panencephalitis, which has recently been recognized as a late complication of congenital rubella infection (23, 24). The CSF showed specific

antibody activity at dilutions through 1:256,000 and had little reactivity against uninfected cells, except at the 1:100 dilution.

Figure 7 depicts the RIA titration of mumps virus antibody in the CSF of a 29-year-old male with meningitis. The patient showed seroconversion to mumps virus CF antigen and a low, stationary serum CF antibody titer to HSV antigen. Significant mumps antibody activity was detected in the CSF through a dilution of 1:4,000.

Figure 8 shows the RIA titration of measles virus antibody in CSF from a 5-year-old male with a clinical diagnosis of subacute sclerosing panencephalitis. Measles antibody was detectable at dilutions through 1:256,000, and the CSF showed little reactivity with uninfected cells.

In Fig. 9 results are shown for an RIA titration of V-Z virus antibody in CSF from an 18-year-old immunosuppressed female with vesicular lesions and CNS involvement. V-Z virus antigen was demonstrated in vesicular lesion material by direct FA staining (21). Specific V-Z CF antibody was detected in the CSF at dilutions through 1:4,000.

Figure 10 presents results of an RIA titration of HSV antibody in CSF from a 25-year-old female with meningitis, who showed a diagnostically significant increase in serum CF antibody to HSV over the course of infection. For the purposes of comparison, a CSF from a meningi-

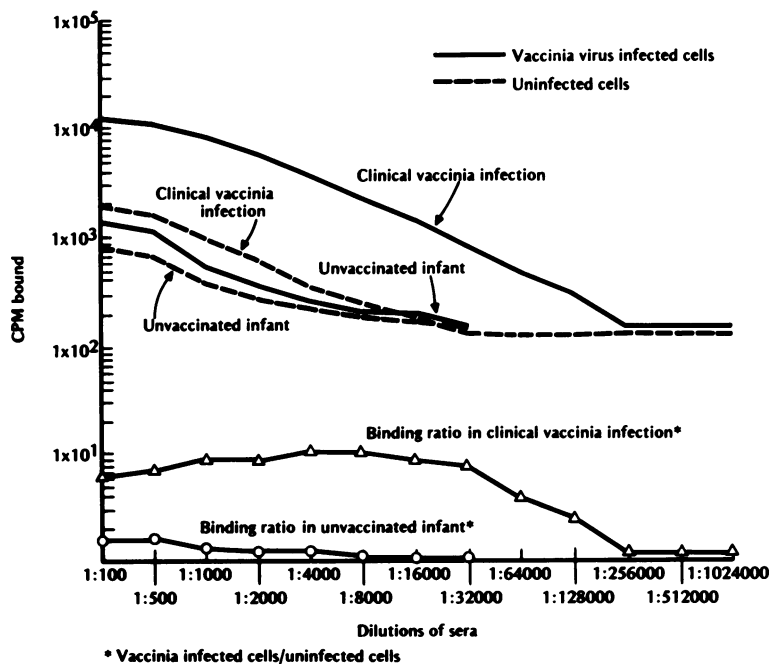


FIG. 5. Titration of vaccinia virus antibody by indirect RIA.

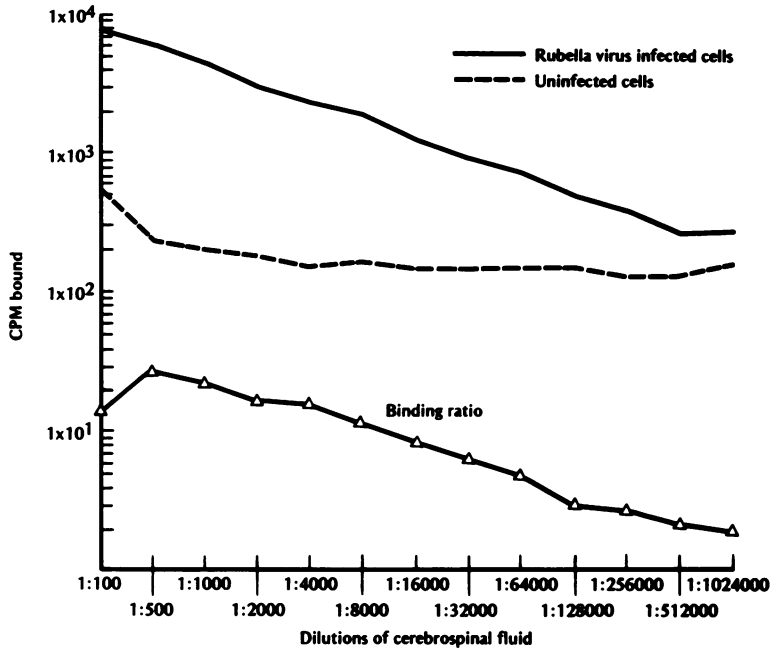


FIG. 6. Titration of rubella virus antibody in cerebrospinal fluid by indirect RIA.

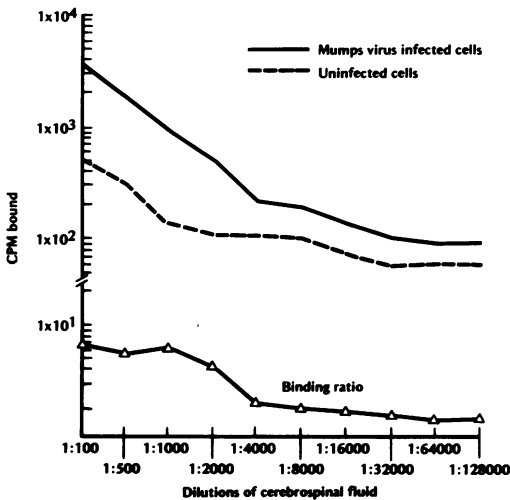


FIG. 7. Titration of mumps virus antibody in cerebrospinal fluid by indirect RIA.

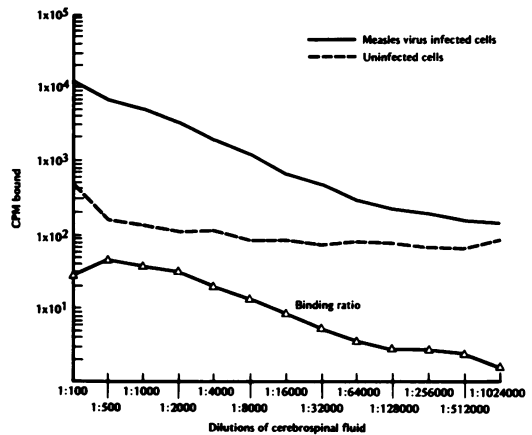


FIG. 8. Titration of measles virus antibody in cerebrospinal fluid by indirect RIA.

tis patient with negative laboratory findings for HSV was titrated in parallel. The CSF from the HSV patient had a homologous antibody titer of 1:4,000.

Comparing the binding curves of antibody in the CSF from the different viral infections, it is seen that curves for rubella and measles antibody in the patients with chronic CNS involvement were similar in that lower CSF dilutions

gave lower specific binding ratios than did higher dilutions, and the CSF antibody showed gradually declining curves, with specific activity persisting through very high dilutions. On the other hand, in the case of mumps, V-Z, and HSV CSF antibody in patients with acute CNS infections, the binding curves declined more sharply with dilution of the CSF, and antibody titers were lower than those for rubella and measles.

Demonstration of CSF antibody to hetero-

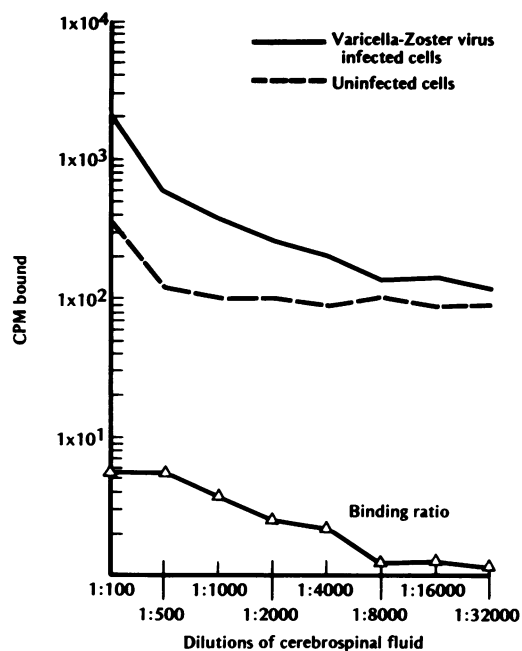


FIG. 9. Titration of varicella-zoster virus antibody in cerebrospinal fluid by indirect RIA.

typic viruses by RIA. The CSF for which homologous antibody titrations are shown in Fig. 6 through 10 were also titrated against certain heterotypic viruses (Table 1). Although some heterotypic antibody was demonstrable in the CSF, titers were markedly lower than those to the infecting virus type, with the exception of the HSV antibody titer in the mumps virus infection. This patient clearly had a current mumps virus infection as evidenced by an increase in serum CF antibody titer from <1:8 to 1:32; CF titers for HSV were 1:32 and 1:16. The patient's CSF also showed a low titer of mumps CF antibody (cf. Table 2), but no CF antibody to HSV.

Comparative sensitivity of RIA and conventional viral antibody assays. Table 2 compares the antibody titers of sera and CSF demonstrated by RIA with those obtained in conven-

tional antibody assay systems. In general, the RIA titers were at least 1,000 times higher than those found by the other assays. The specificity of the RIA test was evidenced by the low antibody titers shown in acute-phase sera and the marked increases in antibody over the course of infection.

DISCUSSION

These studies demonstrate the applicability of an indirect solid-phase RIA system, utilizing fixed virus-infected cells, for titration of serum and CSF antibodies to a variety of human viruses, and they show the RIA system to be markedly more sensitive, in terms of antibody titers detected, than conventional serological tests used for assay of viral antibodies. They also indicate the appropriate dilutions at which

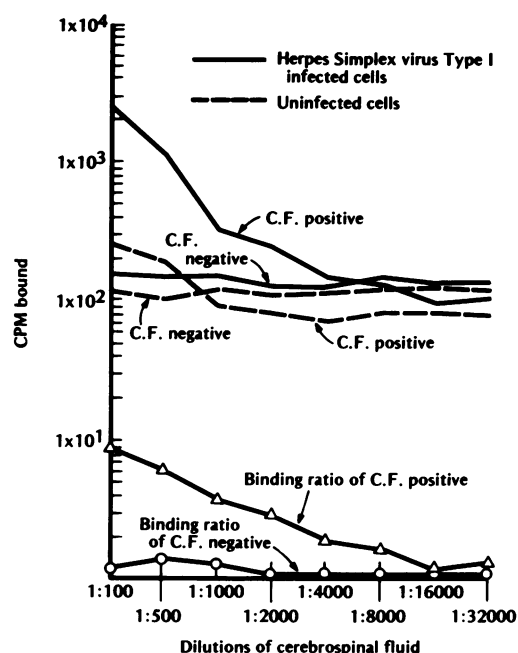


FIG. 10. Titration of herpes simplex virus type 1 antibody in cerebrospinal fluid by indirect RIA.

TABLE 1. RIA titrations of homotypic and heterotypic viral antibody in CSF

Patient	Infecting virus	RIA titer to virus				
		Rubella	Mumps	Measles	V-Z	HSV
JaLo	Rubella	<u>1:256,000</u> ^a	<1:100	<1:100	<1:100	1:100
ArDo	Mumps	1:100	<u>1:4,000</u>	<1:100	1:100	1:4,000
RiEl	Measles	<1:100	<1:100	<u>1:256,000</u>	<1:100	1:2,000
ArRo	V-Z	<1:100	<1:100	<1:100	<u>1:4,000</u>	1:100
ShBa	HSV	1:100	<1:100	<1:100	1:500	<u>1:4,000</u>

^a Underlined numbers are homologous titers.

TABLE 2. Comparison of the sensitivity of RIA with that of conventional viral antibody assays

Test virus	Patient	Specimen	Days after on-set	Antibody titer demonstrated by:				
				CF	HI	Indirect FA	Neutralization	RIA
Rubella	JoJo	Serum	2	<8	<8	— ^a	—	500
Rubella	JoJo	Serum	25	32	256	—	—	256,000
Rubella	JaLo	CSF	6 mo	AC ^b	256	—	—	256,000
Mumps	LiCo	Serum	3	<8	—	—	—	100
Mumps	LiCo	Serum	26	256	—	—	—	256,000
Mumps	ArDo	CSF	19	4	—	—	—	4,000
Measles	MiMc	Serum	1	<8	8	—	—	500
Measles	MiMc	Serum	22	256	4,096	—	—	512,000
Measles	RiE1	CSF	4 mo	32	256	—	—	256,000
V-Z	DrG1	Serum	5	<8	—	—	<8	<100
V-Z	DaG1	Serum	20	256	—	—	512	128,000
V-Z	ArRo	CSF	7	2	—	—	32	4,000
HSV	ShBa	CSF	6	2	—	—	—	4,000
Vaccina	GeD1	Serum	2	—	—	256	512	128,000

^a —, Test not done.

^b AC, Anticomplementary.

sera and CSF could be screened for viral antibody by RIA.

Antibody titers demonstrated in our RIA system were markedly higher than those reported by certain other investigators who used lysates of virus-infected cells adsorbed onto plastic surfaces as a source of viral antigen, and found antibody titers demonstrated by RIA to be only slightly higher than those obtained in conventional viral antibody assays (10, 12, 27). These differences in sensitivity may be due in part to differences in labeling methods (11) and in part to the fact that fixed virus-infected cells, as first utilized by Hayashi et al. (7) for RIA of HSV antibodies, provide a more suitable source of antigen. Viral antigens may be better exposed or preserved in fixed cells than in lysates. Also, fixed cells would be expected to contain all of the antigens produced by the virus, and thus should be capable of detecting antibody against a broad spectrum of virus-specified antigens. Further, it is possible that the competition for adsorbing surfaces of nonviral proteins in cell lysates limits the adsorption of specific viral antigen onto the solid phase of the assay system, and this may result in a less sensitive test. It is pertinent that Cleland et al. (3), using Newcastle disease virus-infected cells fixed with formalin as a source of antigen for RIA of antibody in chicken plasma, and a labeling method similar to ours, obtained titers of the same order as those demonstrated by us for antibodies to human viruses.

Smith et al. (22) have noted that cell destruction by certain highly cytopathic viruses may prevent good fixation and preservation of cell monolayers for RIA. However, using the condi-

tions described for each virus under Materials and Methods, we have had no problems of this sort. It should be emphasized, however, that in using fixed infected cells as a source of viral antigen for RIA, it is essential to standardize the optimal host cell system, conditions for infection, and time of fixation for each virus system, as was done for the present studies. Also, FA staining should show that most of the cells contain viral antigen at the time they are used for RIA.

Cleland et al. (3), in using their RIA for detection of Newcastle disease virus antibody, noted that falsely high antibody titers might be obtained as a result of using the same pipette for preparation of serial serum dilutions. We have also recognized this problem, but have found that the use of a carrier protein (1:5,000 concentration of fetal bovine serum) in the diluent prevents both carry-over of antibody and adsorption of antibody to pipettes and dilution tubes.

Although it has been reported that HSV-infected cells possess receptors for IgG (25), we have seen no evidence of binding of non-antibody IgG by HSV-infected cells, with resultant nonspecific reactivity in the RIA system. Our earlier studies (4, 5) have shown that HSV-infected cells had no greater reactivity than uninfected cells with pre-immunization rabbit or hamster sera or with negative or acute-phase human sera. Further, when we treated HSV-infected cells directly with ¹²⁵I-labeled anti-hamster or anti-human immunoglobulins produced in goats, there was no greater binding of these reagents to infected cells than to uninfected cells. On the other hand, in our RIA

system we have detected binding of non-antibody IgG to cells infected with human cytomegalovirus (6). It would appear that expression of IgG receptors is not so marked in HSV-infected cells as in cytomegalovirus-infected cells. This is also suggested by the fact that numerous workers have used immunofluorescent staining for detection of HSV antigen in infected cells without encountering nonspecific reactivity attributable to IgG receptors, whereas nonspecific, cytoplasmic staining has been generally noted in FA staining of cytomegalovirus-infected cells.

ACKNOWLEDGMENTS

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