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Radioimmunoassay of Measles Virus Antigen and Antibody in SSPE Brain Tissue[1] (40035)

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An indirect solid phase radioimmunoassay (RIA) method developed in this laboratory is highly sensitive for detecting viral antigens in cell cultures and brain tissue, and for assay of viral antibodies in sera and cerebrospinal fluids (1-4). In the present study, the RIA was applied to detection of measles virus antigen in brain tissue of experimentally infected animals and of a patient with subacute sclerosing panencephalitis (SSPE); for detecting specific measles virus antibody in eluates from SSPE brain tissue; and for determining the classes of immunoglobulins represented in the eluted antibody. It was found that RIA could be applied to detection of both measles virus antigen and antibody in the same brain tissue. Elution of brain tissue with chaotropic ions (5) was shown to release greater amounts of specific viral antibody than did elution at an acid pH.

MATERIALS AND METHODS. *Measles virus-infected cell cultures.* The LEC strain of measles virus, isolated from the brain of a child with SSPE (6), was obtained from Dr. H. Koprowski, Wistar Institute. Human fetal diploid lung (HFDL) cells propagated in the bottom of 1-dram glass vials were infected with the virus at a multiplicity of 1, and these were used, as previously described (4), as a source of antigen for assay of measles virus antibody by RIA. Uninfected cells prepared in the same manner were used as controls.

For studies on the adsorption and elution of measles virus antibody to and from infected cells in culture, HFDL cell monolayers in roller bottles were infected with LEC virus at a multiplicity of 1, incubated for 5 or 6 days at 36°. The cells were then dislodged from the bottle surface by shaking with glass beads, sedimented by centrifugation at 2000 rpm for 5 min, washed twice with phosphate buffered saline, pH 7.2 (PBS), and used for the adsorption-elution studies described below.

Measles virus-infected brain tissue. The LEC strain of measles virus adapted to growth in suckling hamster brains was inoculated by the intracerebral route into hamsters 2 to 3 days of age using an inoculum of 0.02 ml containing approximately 1×10^3-1×10^4 pfu of virus. After 3 to 4 days, when the animals first showed signs of illness, the brains were harvested and stored at −70°.

The human brain tissue studied was from a child with SSPE. Upon initial examination in our diagnostic laboratory the brain showed positive fluorescent antibody (FA) staining with measles antiserum. It had been stored at −70° for 8 years at the time of the present study.

Frozen sections from the brain tissue were mounted onto 15 mm round glass coverslips, using five sections per coverlsip without overlapping, and the tissue was air-dried and fixed in cold acetone for 10 min. The coverslips were then transferred to scintillation vials for use in RIA. Adjacent sections from the tissue were mounted on glass slides for FA staining, air-dried, and fixed with acetone.

Measles virus antiserum. Hamsters were immunized with the HNT Philadelphia strain 26 neurotropic virus (7) to produce measles antiserum. This was used as an intermediate serum in the RIA experiments, and also for indirect FA staining.

^{125}I-labeled antibodies to human and hamster immunoglobulins. Heavy chain-specific antisera to human IgA, IgG and IgM produced in goats were obtained from Kallestad Laboratories, Chaska, MN, and goat

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antiserum to hamster gamma globulin from Antibodies, Inc., Davis, CA. These were purified using immunoadsorbents prepared from purified homologous immunoglobulins.

Human IgA was isolated and purified from 400 ml of pooled human serum by the method of Newcomb *et al.* (8). The concentrated IgA was chromatographed twice on 2.5 × 100 cm columns of Sephadex G-200, and the fractions containing IgA were pooled and concentrated to a vol of 2 to 3 ml by ultrafiltration through an Amicon membrane (Amicon Corp., Lexington, MA). Human IgG was purified from 200 ml of pooled human serum, and hamster IgG from 300 ml of normal hamster serum, by three precipitations with 1/3 saturated ammonium sulfate, passage through a DEAE cellulose column, and concentration by Amicon membrane filtration. Human IgM was purified from 600 ml of pooled human serum by the method of Cambier and Butler (9). The concentrated IgM was chromatographed twice on Sephadex G-200 columns and subjected to Amicon membrane filtration. The purity of each human immunoglobulin preparation was confirmed by immunodiffusion against antiserum to whole human serum and against heavy chain-specific antisera to human IgA, IgG and IgM, and the protein concentration was determined by the biuret method. Immunoadsorbents were prepared with the purified immunoglobulins by the method of Avrameas and Ternynck (10) using glutaraldehyde as a cross-linking agent.

IgG fractions of goat antisera to human IgA, IgG and IgM and hamster IgG were added to the homologous immunoadsorbents and incubated at 37° for 30 min followed by overnight incubation at 4° with gentle shaking. After centrifugation at 3,000 rpm for 15 min, the supernatant fluids were removed, and the pellets were washed five times with 200 ml of PBS. The purified IgG was then eluted with 0.1 *M* glycine-HCl, pH 2.8. Eluted IgG was clarified at 3000 rpm for 15 min, the pH was adjusted to 7.0, and the protein concentration was determined by the biuret method after the preparation had been held at 4° overnight.

The purified class-specific immunoglobulins were labeled with ^{125}I by the chloramine T method (11, 12). Complete details of radiolabeling have been published previously (1, 2). For use in the indirect RIA the reagents were diluted in PBS containing 5% normal goat serum to give 40,000 counts/min (CPM) in a volume of 0.1 ml.

RIA of viral antigen and antibodies. Details of the indirect solid phase RIA for detection of viral antigens in infected cells and brain specimens, and for assay of viral antibodies, are given elsewhere (1, 3, 4).

FA staining. The indirect FA staining method used in this laboratory has been described in detail (13).

Elution of antibody from brain tissue. A sample of brain tissue 3 to 4 mm in diameter was triturated with a glass rod in a polycarbonate centrifuge tube. Five milliter of PBS, pH 7.2, was added and the contents of the tube were stirred vigorously on a Vortex mixer, followed by shaking overnight at 4° on a wrist action shaker. After centrifugation at 35,000 rpm for 2 hr at 4°, the supernatant wash fluid was removed and saved for antibody assay. Fresh PBS was added to the tissue, and the above procedure was repeated for a total of five times, or until the wash fluid showed little or no antibody by RIA. Antibody was eluted from the washed brain tissue using either 2.5 *M* KI in PBS (5) or 0.1 *M* glycine-HCl, pH 2.8. Five milliliter of the eluting reagent was added to the tissue, and after thorough mixing and incubation at room temperature for 30 min, the mixture was centrifuged at 35,000 rpm for 2 h at 4° and the supernatant fluid was removed and diluted in PBS containing 1:5000 fetal bovine serum for antibody assay.

Before applying the elution methods to dissociate measles antibody from SSPE brain tissue, their reliability was confirmed using measles-infected HFDL cells and measles-infected hamster brain tissue to which specific antibody was absorbed. To 5 mm square samples of brain tissue or 0.5 ml of infected cell pack was added 0.05 ml of measles immune hamster serum followed, after thorough mixing, by 5 ml of PBS. After incubation for 1 h in a 37° water bath followed by shaking overnight

at 4° the mixture was centrifuged at 35,000 rpm for 2 h at 4°, the supernatant fluid was removed, and the tissue was washed in PBS and eluted as described above. Uninfected HFDL cells and hamster brain tissue were treated in parallel.

Results. Detection of measles virus antigen in brain tissue by RIA. Table I illustrates the specificity and sensitivity of the indirect RIA method for detecting measles virus antigen both in brain tissue of acutely infected hamsters and in the brain of an SSPE patient with a chronic measles virus infection. Controls consisted of brain tissue from uninfected hamsters and a human brain on which attempts to isolate virus or demonstrate viral antigen by FA were negative. In tests with measles-infected tissues the binding ratios obtained by dividing the CPM obtained with measles immune serum by those obtained with normal hamster serum were much above the 2.1 level considered as positive, and in tests with measles-negative brain tissues binding ratios were well below this level.

Of five coverslip preparations examined, each containing five sections of SSPE brain tissue, all were clearly positive by RIA. However, only 16 of 25 individual adjacent sections were positive by FA. These results are not directly comparable, since pools of five sections were examined by RIA, and individual sections by FA, but they do indicate that the method employed for RIA gave positive results with all preparations, while FA examination of individual sections gave negative results in some instances.

TABLE I. DETECTION OF MEASLES VIRUS ANTIGEN IN BRAIN TISSUE BY RIA.

Tissue examined	cpm with intermediate hamster serum (1:4000)		Binding ratio[a]
	Measles virus immune hamster	Normal hamster	
Normal hamster brain	400	339	1.2
Measles-infected hamster brain	3,064	347	8.7
Negative human brain	560	492	1.1
SSPE human brain	1,862	521	3.5

[a] cpm immune serum/CPM normal serum.

Elution of measles virus antibody bound to infected cells and tissues. Table II shows results of preliminary studies designed to determine whether measles virus antibody adsorbed onto infected cells or brain tissue could be effectively eluted and then assayed by RIA. Infected HFDL cells apparently contained greater amounts of accessible measles virus antigen than did infected hamster brain tissue, since greater amounts of antibody were adsorbed, and eventually eluted. Although some antibody was nonspecifically adsorbed to the uninfected cells and brain tissue, this was removed by washing, and none was recovered by elution. With infected cells and tissue, the five wash fluids showed successively less antibody, but large amounts of specific measles virus antibody were released from the washed tissue by elution, and KI was more effective for this purpose than was acid elution.

Efforts were then made to elute viral antibody from the same human SSPE brain in which measles virus antigen was demonstrable by RIA, and also from a human brain from a fatal case of herpes simplex encephalitis. Table III shows in both cases the specificity of the eluted antibody for the infecting virus. Again, KI was more effective than low pH for antibody elution. These results indicate that RIA is applicable to

TABLE II. ABSORPTION AND ELUTION OF MEASLES VIRUS ANTIBODY.

Material treated with measles virus immune hamster serum	RIA titer to measles virus							
	Supernatant fluid after absorption	Wash #1	Wash #2	Wash #3	Wash #4	Wash #5	2.5 *M* KI eluate	0.1 *M* glycine-HCl eluate
Uninfected HFDL cells	400,000	50,000	10,000	2,500	<250	<250	<250	<250
Measles-infected HFDL cells	10,000	5,000	5,000	5,000	2,000	<250	>200,000	100,000
Uninfected hamster brain tissue	400,000	100,000	20,000	20,000	2,500	<250	<250	<250
Measles-infected hamster brain tissue	400,000	100,000	10,000	5,000	5,000	500	100,000	10,000

TABLE III. VIRAL SPECIFICITY OF IMMUNOGLOBULINS ELUTED FROM INFECTED HUMAN BRAIN TISSUE.

Infecting virus	Test virus[a]	RIA titer to test virus: Wash #1	Wash #2	Wash #3	Wash #4	Wash #5	2.5 M KI eluate	0.1 *M* glycine-HCl eluate
Measles[b]	Measles	2,000,000	200,000	100,000	20,000	2,000	100,000	40,000
	HSV	<250	<250	<250	<250	<250	<250	<250
HSV	Measles	2,500	<250	<250	<250	<250	<250	<250
	HSV	25,000	500	500	250	250	25,000	2,500

[a] Infected HFDL cells.
[b] SSPE patient.

the demonstration of both measles virus antigen in the same brain tissue.

Studies on the classes of immunoglobulins present in brain eluates. The first wash fluid and KI and glycine-HCl eluates from the SSPE brain tissue were applied to measles virus-infected cells, and the radiolabeled immune globulins specific for human IgA, IgG and IgM were used to detect antigen–antibody reactions. With each preparation positive results were obtained only with the anti-IgG reagent. The ability of the reagents to detect measles antibody in all three classes of immunoglobulins was confirmed by parallel testing of an early serum from a measles virus infection.

To examine the possibility that high concentrations of IgG antibody in the eluates might be inhibiting reactivity of IgM or IgA antibody, wash fluids and eluates, dialyzed against PBS, were subjected to sucrose density gradient centrifugation, and the gradient fractions were assayed for measles virus antibody by RIA using the heavy chain-specific reagents. As shown in Table IV, only the anti-IgG reagent gave positive results with the fractions. Only IgG was demonstrable in the eluates and gradient fractions by immunodiffusion against antisera to human IgA, IgG and IgM.

As a control on the ability of the gradient to separate viral antibody of different classes, an early serum from a rubella infection was centrifuged in parallel with the wash fluids and eluates, and fractions were assayed for rubella antibody by solid phase RIA using the heavy chain-specific reagents. IgM rubella antibody peaked in fraction 3, IgA antibody in fraction 6, and IgG antibody in fractions 8 and 9.

TABLE IV. CLASS OF MEASLES-SPECIFIC IMMUNOGLOBULIN IN SSPE BRAIN.

Sucrose density gradient fraction[a]	Reactivity in RIA of first wash fluid, KI and glycine-HCl eluates with radiolabeled antibodies to: IgG	IgA	IgM
1	0	0	0
2	0	0	0
3	0	0	0
4	0	0	0
5	+[b]	0	0
6	+	0	0
7	++	0	0
8	+++	0	0
9	++++	0	0
10	+++	0	0
11	+	0	0
12	+	0	0

[a] 1 = most dense, 12 = least dense.
[b] + = antibody titer of 1:1000.
++ = antibody titer of 1:2000.
+++ = antibody titer of 1:5000.
++++ = antibody titer of ≥1:10,000.

Discussion. The present study has extended the use of RIA for detecting viral antigen directly in clinical materials. It has previously been shown that RIA can be used for detecting and typing herpes simplex virus (HSV) directly in human brain tissue (1), and for demonstrating rabies virus antigen in brain tissue (B. Forghani, unpublished). The method can also be used for clear-cut and specific demonstration of measles virus antigen in SSPE brain tissue. The sensitivity of RIA for this purpose was as good as, and perhaps better than, that of FA staining. Further, by using the highly sensitive RIA method for detection of viral antibody in eluates from brain tissue, it was possible to demonstrate measles virus antibody in the same SSPE brain in which antigen was demonstrable.

Assay of viral antibodies in brain eluates by RIA has several advantages, the most notable of which is marked sensitivity, as

evidenced by the high antibody titers obtained. This makes it unnecessary to concentrate eluates for antibody assays (14), and the eluates are diluted >1:100 for antibody assay, making preliminary adjustment of pH or ionic strength unnecessary. The use of highly dilute eluates for antibody detection avoids the problems of nonspecific hemagglutinating or inhibitory activity which has been reported in other studies (14).

The sharp specificity of the RIA for detecting viral antibodies in brain eluates was clearly illustrated by the absence of reactivity of eluates for uninfected brain tissue or from brain tissue infected with a different virus. It is of particular interest that the eluates from the SSPE brain which had high levels of measles-specific activity failed to react in RIA with cells infected with HSV. HSV-infected cells have been reported to produce IgG receptors (15) similar to those produced by cells infected with human cytomegalovirus (16) which bind nonantibody IgG. While IgG receptors on CMV-infected cells may cause some nonspecific binding of IgG in FA reactions (16) and in RIA reactions (3), we have seen no evidence of such nonspecific reactivity in RIA tests with HSV, either in previous studies (1, 2, 4) or the present one.

In this study KI was found to elute two to ten times more viral antibody from brain tissue than was dissociated by low pH. The combination of elution with chaotropic ions and a sensitive RIA for antibody assays may prove useful in studies aimed at identifying a viral etiology of certain chronic neurological infections through the demonstration of specific viral antibody in eluates from diseased tissues.

The highly purified radiolabeled antibodies specific for the various classes of human immunoglobulins demonstrated measles virus antibodies only of the IgG class in SSPE brain eluates. This is in accord with results obtained by Vandvik *et al.* (17) using somewhat different approaches to characterize measles virus antibodies in cerebrospinal fluid and brain extracts from SSPE and multiple sclerosis patients.

Summary. A solid phase radioimmunoassay (RIA) procedure was used to detect measles virus antigen in brain tissue of experimentally infected hamsters and in a human SSPE brain. RIA was also used to detect measles virus antibody in eluates from the same SSPE brain. The antibody in the eluates was sharply measles-specific, showing no reactivity by RIA with HSV-infected cells. Similarly, antibody eluted from a HSV-infected human brain reacted only with the infecting virus. KI eluted two to ten times as much viral antibody from infected brain tissues as was dissociated by low pH. Radiolabeled antibodies to human IgA, IgG and IgM which had been purified and concentrated by immunoadsorption with purified immunoglobulins were used in the RIA system to study the classes of immunoglobulins present in SSPE brain eluates; only measles antibody of the IgG class was demonstrable.

1. Forghani, B., Schmidt, N. J., and Lennette, E. H., Appl. Microbiol. **28,** 661 (1974).
2. Forghani, B., Schmidt, N. J., and Lennette, E. H., J. Clin. Microbiol. **2,** 410 (1975).
3. Forghani, B., Schmidt, N. J., and Lennette, E. H., Infec. and Immun. **14,** 1184 (1976).
4. Forghani, B., Schmidt, N. J., and Lennette, E. H., J. Clin. Microbiol. **4,** 470 (1976).
5. Edgington, T. S., J. Immunol. **106,** (1971).
6. Barbanti-Brodano, G., Oyanagi, S., Katz, M., and Koprowski, H., Proc. Soc. Exp. Biol. Med. **134,** 230 (1970).
7. Burnstein, T., Jensen, J. H., and Waksman, B. H., J. Infec. Dis. **114,** 265 (1964).
8. Newcomb, R. W., Normansell, D., and Stanworth, D. R., J. Immunol. **101,** 905 (1968).
9. Cambier, J. C., and Butler, J. E., Prep. Biochem. **4,** 31 (1974).
10. Avrameas, S., and Ternynck, T., Immunochem. **6,** 53 (1969).
11. Hunter, W. M., and Greenwood, F. C., Nature (London) **194,** 495 (1962).
12. McConahey, P. J., and Dixon, F. J., Int. Arch. Allergy Appl. Immunol. **29,** 185 (1966).
13. Schmidt, N. J., Lennette, E. H., Woodie, J. D., and Ho, H. H., J. Lab. Clin. Med. **66,** 403 (1965).
14. Weil, M. L., Leiva, W. A., Heiner, D. C., and Tourtellotte, W. W., J. Immunol. **115,** 1603 (1975).
15. Westmoreland, D., and Watkins, J. F., J. Gen. Virol. **24,** 167 (1974).
16. Furukawa, T., Hornberger, E., Sakuma, S., and Plotkin, S. A., J. Clin. Microbiol. **2,** 332 (1975).
17. Vandvik, B., Norrby, E., Nordal, H. J., and Degré, M., Scand. J. Immunol. **5,** 979 (1976).

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