Solid Phase Indirect Radioimmunoassays for Rapid Diagnosis of Sindbis Virus Antigen

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SUMMARY

Indirect radioimmunoassays have been developed for the rapid detection of Sindbis virus. Dilutions of Sindbis virus from tissue culture fluids have been immobilized and allowed to react with rabbit anti-Sindbis virus antibodies. The bound antibodies were assayed either by 125I-labelled anti-rabbit IgG-antibodies or alternatively by addition of human complement and 125I-labelled anti-human C1q antibodies or 125I-labelled protein A.

The aim of a rapid virus diagnosis should be to give answer to the aetiology within 2 to 3 h (Gardner, 1977). A rapid solid phase radioimmunoassay in microtitre plates for the measurement of antiviral antibodies was developed by Rosenthal et al. (1973). Several authors (Blechschmidt et al. 1977; Crawford et al. 1977; Yung et al. 1977) applied this method for the detection of virus antigens in direct and indirect test systems. The application of an indirect radioimmunoassay using labelled antibodies against IgG bound to antigen offers an advantage since only one serum has to be labelled for the identification of an antigen provided that the specific antisera to be tested were obtained from animals of the same species. These requirements cannot be realized in every case. Therefore, the aims of the present investigations were to develop sensitive indirect radioimmunoassays for the determination of Sindbis virus antigens which are independent of the source of immune sera. The methods make use of two possibilities, either the binding of the complement component C1q to the antigen-antibody complexes (Müller-Eberhard, 1975), or the strong affinity of protein A from Staphylococcus aureus for the Fc region of most classes of IgG (Kronvall et al. 1970).

Sindbis and Semliki Forest viruses were propagated in BHK cells. The supernatants of infected cells containing 1.5 x 10^9 infectious particles per ml were stored frozen at -20 °C until they were assayed. Antiserum against Sindbis virus was produced in rabbits using the method described by Mussgay & Rott (1964). Nonspecific antibodies were removed by absorption with BHK cells. Anti-rabbit IgG serum was purchased from Behring (Marburg, West Germany) and anti-human-C1q serum was purchased from Behring (Marburg, West Germany). Protein A was obtained from Pharmacia (Uppsala, Sweden). IgG and protein A were iodinated with 125I (Buchler, Braunschweig, West Germany) by the chloramine-T method as described by Greenwood & Hunter (1963). Separation of the labelled protein from the unreacted NaI was carried out by gel filtration using Sephadex G-25 (PD 10, Pharmacia).

For the indirect solid phase radioimmunoassay virus suspensions were serially diluted in 0.1 M-phosphate buffer (pH 7.6) containing 0.1 % bovine serum albumin. Twenty μl portions of each dilution of virus were added to the wells of a flexible microtitre plate (Flow, Bonn, West Germany) and the plate was then dried in a stream of warm air. The wells were washed first with distilled water and then with PBS and 20 μl of anti-virus IgG in a constant 1:1000 dilution containing 0.1 % bovine serum albumin were then added. (As a source of
Fig. 1. The indirect solid phase radioimmunoassay for virus antigens. Sindbis virus adsorbed to the wells of a microtitre plate in serial dilutions ($10^{-4}$, $5 \times 10^{-4}$, $2.5 \times 10^{-4}$, $10^{-4}$, $5 \times 10^{-5}$, $2.5 \times 10^{-5}$, $10^{-5}$, $5 \times 10^{-6}$, $2.5 \times 10^{-6}$, $10^{-6}$, $5 \times 10^{-7}$) was coated with IgG from rabbit by hyperimmune serum. Binding of antibody to immobilized antigen was determined by a labelled antiglobulin. o---o, Semliki Forest virus used as a control. (b) Antigen–antibody reaction in the presence of complement was measured by a second labelled antibody against C1q-component of complement. (c) The presence of antibodies bound to Sindbis virus was detected by labelled protein A. The dashed lines demonstrate the level of background. The mean of four titrations is shown in every diagram. The highest standard deviation of individual titrations was 5.6\%.

complement, human serum in tris-NaCl buffer containing EDTA was added in some instances; in these cases the same buffer was used for washing.) The microtitre plates were covered and left at 37 °C for 1 h and afterwards washed five times with PBS containing 0.1% bovine serum albumin. Then 25 μl, containing about 15000 ct/min of the second reactant (+0.1% bovine serum albumin) labelled with $^{125}$I (anti-rabbit IgG, anti-human-C1q or protein A), were added to the wells followed by a further incubation at 37 °C for 30 min. Separation of bound and free radioactivity was accomplished by decanting the free counts while the bound remain adsorbed. The plates were washed five times with PBS and dried as described above. The wells of the plates were cut out and the radioactivity was determined directly in a Packard auto gamma spectrometer.
In the assay using $^{125}$I-labelled antibodies against rabbit IgG, the amount of antibodies bound to serially diluted Sindbis virus antigen was determined by adding the labelled anti-rabbit IgG antibodies as described above. The results are shown in Fig. 1(a). Variations in antibody concentration did not uniformly affect the configuration of the curve. At high antibody dilutions decreased binding to antigen was observed over the entire curve, but was more pronounced at the peak (results not shown). The specificity of the assay was shown by using Semliki Forest virus as a control.

Fig. 1(b) shows that binding of anti-Sindbis virus antibodies to immobilized Sindbis virus in serial dilutions in the presence of EDTA-treated human serum could be detected by a labelled antibody against human C1q. The arrangement of the experiment was similar to that shown in Fig. 1(a), with the exception of the presence of human serum (1:50) as a source of C1q in the anti-virus antibody solution.

The binding of antibodies to immobilized Sindbis virus in a radioimmunoassay could also be detected by a labelled preparation of protein A. Since protein A binds to IgG the appearance of radioactivity demonstrates antibodies which are bound to the immobilized antigen in the microtitre wells. In Fig. 1(c) the results of such an experiment are demonstrated. The dilution profile of Sindbis virus was comparable with those shown in Fig. 1(a) and (b).

The results indicate that the assay in each of the three methods requires the antigenic mass of at least 500 to 1000 infectious units of Sindbis virus as antigen and the specific decline of the curve starts at an antigen-dilution of about $10^{-5}$. Infectivity, rather than protein concentration, was selected as the more reliable estimate of equivalent antigenic mass, since it is crude but not purified material which has usually to be determined by diagnostic procedures. Therefore, crude supernatants of infected cells provided the basis for rapid virus diagnosis. If smaller amounts of virus antigens are present in the undiluted material the specific decline of the curve will start at lower dilutions. Nevertheless a quantitative analysis of the virus which has to be diagnosed was not attempted because of the difficulties in correlating infectivity and antigenic mass.

The specificity of the reaction was shown by using as a control Semliki Forest virus coated with anti-Sindbis virus IgG. The highest percentage of radioactivity bound in each assay-system was not more than 32% (shown in Fig. 1a as an example) compared with the specific binding obtained by using Sindbis virus.

In the present study it was found that at different antigen dilutions the amount of specific antibodies bound to the immobilized antigen varies with the decreasing concentration of antigens, thus resulting in a peak. Therefore, antigen must be present in an optimal concentration, otherwise sterical hindrance of binding may vitiate the results. These prerequisites are obtained by the serial dilution of antigens used in this study.

In a preliminary experiment (results not shown) West Nile virus could be identified with both systems using specific goat antiserum. It will be the aim of further work to show that immune sera from different species can be used for the detection of other virus antigens using the anti-complement system and the protein A system.

The procedures described in this study may have potential value for rapid diagnosis of viruses which can be completed within 3 to 4 h.

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Federal Research Institute for Animal Virus Diseases, P.O. Box 1149, D-7400 Tübingen, Federal Republic of Germany

P.-J. ENZMANN
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