The Sensitivity of Measles Virus
Haemolysin to Acetone and the Preparation of Mono-specific
Human Anti-Haemolysin by Absorption

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SUMMARY

The haemolysin of measles virus, either in the virion or in infected cells, is functionally and antigenically sensitive to acetone. Absorption of human sera with acetone-treated, measles virus-infected cells removes antibodies to all measles virus structural antigens except haemolysin. The antibody titres of absorbed sera give good correlation in HLI, neutralization and fluorescent antibody staining on unfixed infected cells.

Recent methods of preparation of antisera to measles virus haemagglutinin and haemolysin do not provide antisera that are recognizably monospecific. Such antisera are required to identify measles virus polypeptides. We describe here a useful indirect method of acquiring mono-specific anti-haemolysin to measles virus.

The main properties associated with measles virus in vitro are haemagglutination, haemolysis, cell fusion, haemadsorption and infectivity which can be assayed by plaque formation. In cells infected with measles virus, two envelope antigens are expressed at the surface, haemagglutinin (HA) and haemolysin (HL) (Ehrnst et al. 1974). Measles virus haemagglutinin and other virus structural antigens in infected cells are resistant to acetone fixation whereas virus haemolysin is destroyed antigenically by such treatment (Fraser et al. 1978). Thus HA and HL on the surface of infected cells can be studied separately by fluorescent antibody methods on unfixed and acetone-fixed monolayers. We have found that most human sera contain higher titres of haemolysin-inhibiting antibodies (HLI) than haemagglutinin-inhibiting antibodies (HAI) (Fraser et al. 1979).

The difference in sensitivity to acetone of the measles virus antigens, HA and HL, on infected cells could have been a secondary effect due to the effect of the acetone on the cell membrane. It was therefore necessary to test the effect directly on measles virions. Extracellular virus, concentrated by centrifugation at 73,000 g for 90 min at 4 °C and resuspended in a small vol. of 0.01 M-phosphate buffered saline (PBS), pH 7.4, was treated with 1%, 5%, 10% and 25% (v/v) acetone in PBS for 30 min at 4 °C. The acetone was removed by dialysis overnight at 4 °C and the samples were assayed for HL, HA (Norrby & Gollmar, 1972; Shirodaria et al. 1976) and infectivity by plaque titration (Gould, 1974). The treatment of virus preparations with 1%, 5% and 10% acetone had no effect on HL or HA but treatment with 25% acetone destroyed haemolysin (titre: 64 reduced to < 2 per 0.25 ml after dialysis) and infectivity (titre: 3 x 10⁸ p.f.u. reduced to nil or non-infectious after dialysis) but had no effect on HA (titre before and after dialysis: 64 per 0.25 ml).

The differing sensitivity of measles virus HL and HA to acetone and the existence of separate HLI and HAI antibodies in most human sera (Norrby & Gollmar, 1975) provide a means of testing differential absorption by cell preparations. Paraformaldehyde fixation was used as a control. Measles virus-infected cell packs were prepared by infecting Vero or HEp2 monolayers with the Edmonston strain of measles virus (Gould, 1974) at a multiplicity of 0.04. After 48 h at 37 °C the cells were scraped from the glass and washed twice in PBS, pH 7.4. They were then treated with acetone for 10 min at room temperature.
Table 1. Absorption of human sera with treated measles virus-infected Vero cells

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<thead>
<tr>
<th>Absorbed with:</th>
<th>Serum</th>
<th>HAI</th>
<th>HLI</th>
<th>F.A. staining acetone-fixed cells</th>
<th>F.A. staining live cells</th>
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<td>Acetone-treated virus-infected cells</td>
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<td>Paraformaldehyde-treated virus-infected cells</td>
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<td>Paraformaldehyde-treated uninfected cells</td>
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Paraformaldehyde-treated uninfected cells

The results with seven human sera (Table 1) showed clearly that absorption with acetone-treated, measles virus-infected cells removed HAI antibody but did not affect titres of HLI antibody. Immunofluorescence showed staining by these absorbed sera of antigen at the membrane of unfixed infected cells (Fig. 1a) but failed to stain acetone-fixed infected cells (Fig. 1b). It is interesting to note that in the same serum the titres of HLI antibody were similar to fluorescence titres on unfixed virus-infected cells. Absorption of two sera with paraformaldehyde-fixed measles virus-infected cells removed HLI, HAI antibodies and fluorescent staining was not observed on either unfixed or acetone-fixed infected cells (Table 1). Control absorptions with paraformaldehyde-fixed uninfected Vero cells had no effect on HLI, HAI or fluorescent antibody titres (Table 1). The fluorescent staining by such treated sera showed membrane staining on unfixed infected cells (Fig. 1c) and intracellular staining of antigens on acetone-fixed infected cells (Fig. 1d). Clearly, the acetone treatment destroys the ability of haemolysin to absorb its antibody. The effect was confirmed by HLI, neutralization and fluorescent antibody when absorptions with virus-infected and uninfected cells were carried out with hyperimmune monkey anti-measles serum.

Specificity of the results was further confirmed by removing HA from the cell surface by treatment with trypsin (Ehrnst & Sundqvist, 1975; Fraser et al. 1979). The unabsorbed and absorbed sera titrated on untreated and trypsin treated HEp2 cells chronically infected with...
measles virus gave exactly the same fluorescence titres. The virus neutralization test (Shirodaria et al. 1976) showed that absorbed serum, containing no HAI antibody, was able to neutralize the virus and neutralization titres of unabsorbed and absorbed sera were similar to HLI antibody titre and immunofluorescence titres on unfixed infected cells. For example, a human serum before and after absorption had neutralization, HLI and immunofluorescence titres on unfixed infected cells of 320, 320 and 640 respectively. The absorbed serum was found to precipitate a single polypeptide in the radioimmunoprecipitation test (M. Gharpure, personal communication).

Our results show that antigenically and functionally, measles virus HA is insensitive to acetone whilst HL is sensitive. Thus absorption of human serum with acetone-treated measles virus-infected cells provides a simple and useful method for the preparation of mono-specific antiserum to measles virus haemolysin.

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REFERENCES


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