Detection of Lymphocytic Choriomeningitis Virus Antibody in Murine Sera by Immunofluorescence

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An immunofluorescence technique developed for detection of antibody in murine sera to lymphocytic choriomeningitis virus is described.

Lymphocytic choriomeningitis (LCM) virus infection of laboratory mice is reported to be widespread (6). In addition to endangering personnel, unrecognized infection in colonies can introduce diagnostic and experimental error. Serological surveillance, however, has been limited to the complement fixation (CF) test, since mice may not form neutralizing antibody to the virus (7). This report describes an indirect fluorescent-antibody (FA) technique for detection of LCM virus antibody in murine sera.

To obtain anti-mouse globulin for conjugation, rabbits were immunized with globulin from mouse sera fractionated by (NH₄)₂SO₄ solution at a final concentration of 50% saturation. Sera from the immunized rabbits were fractionated in the same manner, and recovered globulin was conjugated to fluorescein isothiocyanate under conditions that resulted in a final product containing 7 μg of fluorescein per mg of protein (4).

LLC-MK₂ cell (3) monolayers were used to produce antigen for staining. Four days after inoculation with the Armstrong E-350 strain of LCM virus obtained from the American Type Culture Collection, cells were scraped from their container wall, washed twice with isotonic phosphate-buffered saline (PBS; pH 7.6), and allowed to dry on glass slides. The dried films were acetone-fixed for 10 min at room temperature. They remained satisfactory when stored at −60°C for 2 months, the longest period tested.

Individual sera for examination were collected from ether-anesthetized mice by severance of the jugular artery (5) 20 days after intraperitoneal inoculation with virus. For FA titrations, the infected LLC-MK₂ cell films were exposed for 20 min at room temperature to serial dilutions of serum in PBS, washed thoroughly with the buffer, and allowed to dry. Undiluted conjugate was then applied. After 20 min, the cells were washed as before and mounted with buffered glycerol for fluorescence microscopy. CF tests were performed by the microtiter technique (1).

In Table 1, FA and CF titers of LCM virus antibody are compared for 52 mouse sera. FA titers were twofold lower than CF titers with 4%, equal with 8%, twofold greater with 29%, and fourfold greater with 59% of the sera. To establish the specificity of the staining, substitution of sera from uninfected mice, conjugated globulin from unimmunized rabbits, or uninfected LLC-MK₂ cells resulted in negative reactions. Also, sorption of positive sera with LCM virus-infected mouse brain eliminated staining unaffected by sorption with uninfected brain. Finally, inhibition of staining by dilution of immune conjugate with unlabeled anti-mouse globulin was clearly greater than by dilution with globulin from unimmunized rabbits.

Employing conjugated anti-human globulin, Cohen et al. (2) used immunofluorescence to examine human sera for LCM virus antibody. The present report demonstrates the sensitivity of the FA technique for antibody to the virus in murine sera. If congenital infection in colonies is suspected, virus isolation should be attempted, since immunological tolerance may interfere with serological response. To detect introduction of the virus into colonies, however, the indirect FA technique makes feasible the routine examina-

### Table 1. Comparison of CF with FA titers of murine sera for LCM virus antibody

<table>
<thead>
<tr>
<th>CF titer</th>
<th>FA titer</th>
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<tbody>
<tr>
<td>20⁺</td>
<td>40 (3)</td>
</tr>
<tr>
<td>40</td>
<td>20 (1), 40 (1), 80 (3), 160 (8)</td>
</tr>
<tr>
<td>80</td>
<td>80 (2), 160 (6), 320 (18)</td>
</tr>
<tr>
<td>160</td>
<td>80 (1), 160 (1), 320 (3), 640 (2)</td>
</tr>
</tbody>
</table>

*Reciprocal of serum dilution. Number of individual sera.
tion of sufficiently large numbers of sera for adequate sampling.

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LITERATURE CITED


