Diagnosis of Junin Virus in Cell Cultures by Immunoperoxidase Staining

Brief Report

By

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With 1 Figure

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Summary

Vero cells grown in Leighton tubes were infected with blood taken from Argentine Hemorrhagic Fever patients. In 11 out of 12 cases, and between the 2nd—8th days p.i. of the monolayers, Junin viral antigen was detected by the PAP method.

In the present study, the peroxidase/antiperoxidase (PAP) method was applied to the identification of Junin virus, the etiological agent of Argentine Hemorrhagic Fever. Preliminary experiments were made in which several reference strains [XJ-Clon3 (3), Gar (4) and Mones Cazón (9)] and cell cultures of various kinds (primary cultures of rabbit kidney, of mouse fibroblasts and of mouse brain; Vero and BHK-21 cell lines) were tested. Once the method was thus standardized, the procedure was applied for the virological diagnosis of Argentine Hemorrhagic Fever. Twelve samples of heparinized blood taken from twelve patients during the acute phase of the disease, were inoculated layering 0.1 ml of blood onto Vero cells grown in Leighton tubes. Cell cultures were harvested at daily intervals up to 8 days p.i. The unlabelled antibody enzyme (PAP) method was used (2, 8) according to the following sequence: 1. Washing of the coverslips with Tris-buffered saline (TBS), 0.05 M, pH 7.6; 2. Fixation with a mixture of methanol and crushed dry ice for 2 minutes; 3. Several washings with TBS to which 1 per cent normal goat serum had been added (TBSG); 4. Anti-Junin rabbit-antiserum was diluted 1:50 and then applied overnight at 4°C; 5. Application of goat anti-rabbit IgG antiserum (Cappel Lab., Downington, Pa., U.S.A.), diluted 1:100, during 30 minutes at room temperature; 6. Application of rabbit-produced PAP (Cappel Lab.), diluted 1:100, during 30 minutes at room tempera-
ture. TBSG was employed for dilution of the three antisera. The monolayers were thoroughly washed with TBSG after the application of each antiserum. The detection of peroxidase was done under microscopic control with diluted (0.03 per cent) 3,3′ dianobenzydine-tetrahydrochloride (Fluka AG, Chemische Fabrik, Buchs, Switzerland) plus 0.05 per cent hydrogen peroxide. After washing with distilled water, each preparation was lightly stained with Mayer’s hematoxylin and mounted. On some occasions this nuclear counterstaining was omitted.

In the preliminary experiments, immuno-labelling was attained in all the cellular systems tried out as well as with all the viral strains utilized for infection. The positive PAP-stained cells showed their cytoplasm filled with dark-orange granules, which occupied both the perikaryon and the cytoplasmic processes. These cells stood out sharply from the non-infected cells. The observations of positive immuno-labelled cells always coincided with the presence of infective Junin virus in the supernatant. The best observations, both in early appearance and in contrast, were obtained using the Vero cell line and XJ-Clon 3 strain; in such system, Junin antigen could be detected as soon as 24 hours p.i. of the monolayer (Table 1).

Table 1. Intensity of positive PAP reactions in Vero cells infected with varying dilutions of XJ-Clon 3 strain of Junin virus

<table>
<thead>
<tr>
<th>Virus input</th>
<th>Hours post-infection</th>
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<tbody>
<tr>
<td>LD₅₀</td>
<td>24  48  72  96</td>
</tr>
<tr>
<td>10⁴</td>
<td>++       ++       ++   ++    ++</td>
</tr>
<tr>
<td>10³</td>
<td>+        ++       ++   ++    ++</td>
</tr>
<tr>
<td>10²</td>
<td>0        +        +     +     +</td>
</tr>
<tr>
<td>10¹</td>
<td>0        0        0     0     0</td>
</tr>
</tbody>
</table>

Specific immuno-labelling was also attained in 11 out of 12 samples tested on Vero monolayers. Positive results were observed on the 2nd day p.i. of monolayers in 2 cases; on the 3rd day in 1 case; on the 4th day in 5 cases; on the 6th day in 1 case; on the 7th day in 1 case; and on the 8th day in 1 case. Immuno-labelled cells appeared isolated or in clusters (Fig. 1). Occasionally, the reaction was scanty so that finding positive cells required careful examination of the whole monolayer. However, these few positive cells were so characteristic that the diagnosis did not leave any doubt (Fig. 1, arrows).

Fixation of tissues is of primary importance for PAP technique staining. It is known that one of the best fixatives for use with this technique is Bouin’s fluid. Excellent immuno-labelling of type 1 Herpes simplex virus antigen in suckling mouse tissues (5) was obtained using this fixative. However, when dealing with Junin antigen, no staining was obtained when Bouin’s fluid was used, in spite of trying several changes in the PAP technique and in the time of action of the fixative. Positive results were only achieved when ethanol, methanol, ether, acetone, or chloroform were used. Methanol proved to be the best, but another technical refinement was found to be essential; this consisted of carrying out the fixation at very low temperature (done by adding small pieces of dry ice) in order to obtain the most intense and precise staining.