Brief Communication

Growth Curves of Rauscher and Friend Murine Leukemia Viruses in JLS-V9 Tissue Culture

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Continuous replication of the Rauscher and Friend murine leukemia viruses in tissue culture has been described (3, 8, 12, 13, 16—18). However, except for the work of Osato et al. (12) and Chopra and Shibley (2), little has been done to demonstrate in vitro growth curves of these viruses analogous to the in vivo work described by Rauscher and Allen (14). This communication describes studies in which an attempt was made to develop such in vitro growth curves using an established mouse bone marrow cell line (JLS-V9). These cells were used since they were previously shown to be free of contaminating agents, as well as susceptible to infection with the Rauscher virus (18).

Both Rauscher (10^5.5 ID_{50}/ml) and Friend (10^6.8 ID_{50}/ml) mouse plasma viruses were each concentrated to 10 gm equivalents according to Moloney's method (11) and diluted 1:10 in Eagle's minimum essential medium containing 10% calf serum (E_{10}C_{10}). One ml of each cell-free virus inoculum was separately incubated with an equal volume of a JLS-V9 cell suspension containing 1.2 x 10^6 cells per ml. Virus attachment was allowed to proceed in a 37°C water bath for two hours. The cells were kept in suspension by gentle agitation every 15 minutes. After adsorption the residual virus and infected cells were diluted to 10 ml and the cells sedimented by low speed centrifugation (1500 rpm for 5 minutes). This was followed by a series of five washes, two with E_{10}C_{10}, then one containing 2.5% Rauscher virus immune rabbit serum, and finally two more washes with E_{10}C_{10}. The immune serum was used in an effort to neutralize residual unadsorbed virus from the infected cells. This serum was shown previously to have virus neutralizing activity (B. S. Wright, unpublished data) and could provide 100% protection in mice inoculated with a 10^{-1} dilution of the Rauscher mouse plasma virus. Although the neutralizing activity of this serum for the Friend virus was not determined, the cross-reaction between the two viruses has been well established (4, 5, 10).

The third wash of the control noninfected V9 cells contained 2.5% normal rabbit serum. Although residual virus was not expected, the supernatants from the final wash of the infected cells were concentrated 20-fold by centrifugation at 40,000 x g for one hour in a model L ultracentrifuge. When these concentrates were stained by the negative staining technic (1) and examined in the electron microscope, virus was not seen.

After the last wash, each of a series of T-60 flasks was seeded with 8 x 10^6 viable cells per flask. On Day 3 and every 2 days thereafter, one flask was removed from the incubator and the tissue culture fluid clarified by low speed centrifugation. The supernatants were recenterfuged at 40,000 x g for one hour before resuspending the pellet to a 20-fold concentration of the original volume of tissue culture fluid. All concentrates were examined for virus particles by the negative staining technic. In addition to the particle counts, each concentrate of pelletized tissue culture fluid was assayed for infectious virus by inoculating 0.1 ml samples intraperitoneally into weaning BALB/c mice. The infected cells were also sectioned and examined by electron microscopy for the presence of budding virus. The electron microscopy (EM) method of estimating virus at any given time is at best a rough evaluation of the total viral yield. However, it does not estimate the amount of leukemogenic or nonleukemogenic virus. Conversely, in vivo mouse titrations do not estimate the amount of nonleukemogenic virus present at any given time. Furthermore, Levy et al. (9) have shown that over 90% of a mouse plasma virus population is inactivated in 1—2 days at 37°C in a tissue culture medium containing 15% serum. For these reasons, EM was used as an estimate of the total virus yield, while inoculation of the 20 x concentrates determined if any leukemogenic virus was present.

Chart 1 illustrates graphically the electron microscope data shown in Table 1 for the 20-fold tissue culture concentrates of Rauscher and Friend virus-infected JLS-V9 growth medium. All virus concentrates were diluted 1:3 with 2% phosphotungstic acid at pH 4.6 before examination in the electron microscope. The virus counts of these diluted samples are plotted along the ordinate and were approximated using a 200-mesh electron microscope grid (2). As shown in Chart 1, virus was initially observed in the 20-fold concentrated tissue culture fluids 5 days following infection and thereafter.

Typical Rauscher or Friend virus particles were never seen when control cells were examined by the thin sectioning technic or when concentrated supernatant fluids were negatively stained.

As early as 3 days postinfection, some evidence of virus budding was observed in thin sections of the Rauscher virus-
under similar conditions has again revealed the early release of infectious virus for BALB/c mice. The Friend virus-infected cells were not carried beyond the length of the experiments reported here.

The Friend virus growth curve is similar to the one reported by Osato et al. (12), in which infectious virus was detected in tissue culture fluids from HA/ICR Swiss mouse embryo monolayers 5 days after infection. They also observed a specific immunofluorescence in the virus-infected cells as early as 3 days postinfection. In addition, Sidwell et al. (15) reported that they could demonstrate the presence of the Friend virus in Swiss mice on the third day after virus inoculation. These results agree with those reported in the present study. However, results with the Rauscher virus growth curve differ from those reported by Chopra and Shibley (2). They first detected the Rauscher virus from JLS-V9 inoculated as monolayers with either tissue culture (JLS-V5) or mouse plasma virus 10 days after infection. The same pattern of viral multiplication was observed when the monolayers were infected with six times as much virus as was previously reported (2). When 10 gm equivalents Rauscher virus was diluted 10,−1 10,−3 and 10,−5 and used as inoculum for V9 cells in suspension, extracellular virus was detected in the tissue culture fluids after 5, 9, and 16 days respectively.

Although the appearance of virus 9 days after infection (from 10,−5 inoculum) resembles the growth patterns previously described, this inoculum was 200-fold less concentrated than that used by Chopra and Shibley (2). These results suggest that they had surpassed the optimum virus to cell ratio at the time of adsorption, and interference resulted in a delay of virus production (7). These results also confirm those of Hartley et al. (6) in which high doses of virus and infection of cells in suspension produce detectable complement fixation antigen within 6 days and also tend to support the viral nature of the antigen.

The in vitro growth curves of the two murine leukemia viruses described in this report are quite similar to the in vivo growth curve of the Rauscher virus (14). They are also analogous to the curves of many nontumorigenic viruses. Under the conditions of this study, both viruses were detected early after infection of the V9 cells, indicating a rapid synthesis and progressive increase of extracellular virus. In these experiments,

Table 1

<table>
<thead>
<tr>
<th>Days after infection</th>
<th>Electron microscopy</th>
<th>Rauscher virus</th>
<th>In vitro assay*</th>
<th>Friend virus</th>
<th>In vitro assay</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>8-fold concentrate</td>
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<td></td>
<td>Cells</td>
<td></td>
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<td>F/T</td>
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<td>9/13</td>
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<tr>
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<td>9/12</td>
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<td>12</td>
<td>ND</td>
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*In vitro and in vivo detection of Rauscher and Friend virus from infected JLS-V9. ND, not done; P/T, number of mice with palpable spleens/total number of mice; D/T, number of mice dead with leukemia/total number of mice inoculated.

a Observation period, 4 months.

b The existence of budding virus particles.

c Virus particles/grid square (200-mesh grid).
maximum titers were reached within 10 days. In a repeat experiment with the Rauscher virus, this peak was reached in seven days.

The results of these experiments demonstrate the susceptibility of the JLS-V9 cells to both the Rauscher and Friend viruses. The control cells are also readily infected with the Moloney virus (18).

The experimental results presented here indicate that the JLS-V9 cells provide an excellent host cell system for studies of the murine leukemia viruses.

Acknowledgments

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Addendum

Since completion of this study, mouse titrations of similar tissue culture samples have shown a peak of virus leukemogenicity in seven days.

References

Figs. 1-3. Typical murine leukemia virus buds in infected JLS-V9 cells. Fig. 1, 3-day Rauscher virus bud, × 113,400. Fig. 2, 12-day Rauscher virus buds with some C-type extracellular virus, × 63,000. Fig. 3, 12-day Friend virus buds, × 63,000.
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