Studies on the Biological and Antigenic Properties of ESP-1 Type C Virus Particles

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SUMMARY

ESP-1 virus provides a helper function to defective Friend spleen focus-forming virus (SFFV). This helper activity is demonstrable only in B-type mice (Fv-1b) under conditions in which the N-tropic lymphatic leukemia-inducing virus contained in the Friend virus complex is inhibited. ESP-1 virus therefore expresses a B-tropic host range in vivo with respect to its ability to provide a helper function to defective SFFV. Further, this helper activity is directly associated with the presence of the ESP-1 virus since activity was recovered only from gradient samples ranging from 1.15 to 1.17 g/ml. All samples contained ESP-1 virus and Mycoplasma. However, Mycoplasma was derived from the ESP-1 cell line and maintained in broth culture only enhanced SFFV titer estimates and failed to provide a helper function to SFFV. ESP-1 virus helper activity is dose dependent and heat labile and is neutralized by rat antisera directed against both whole and Tween:ether-disrupted ESP-1 virus. Further, ESP-1 virus is not neutralized by type-specific murine antisera directed against both the Rauscher virus complex and the lymphatic leukemia-inducing helper virus component of the Rauscher virus complex and Moloney leukemia virus. These findings, together with the observations that these same murine antisera neutralized human-embryonic-kidney-cell-grown Rauscher leukemia virus, suggest that the type C virus designated ESP-1 has some of the biological properties of the murine leukemia viruses (i.e., helper activity for the induction of leukemia in mice) but does not contain type-specific envelope antigen(s) characteristic of Moloney leukemia virus, N-tropic Friend lymphatic leukemia-inducing virus, or human-embryonic-kidney-cell-grown Rauscher leukemia virus.

INTRODUCTION

Since the observation of type C particles in a monolayer cell culture (ESP-1) established with the pleural effusion cells of a child with Burkitt lymphoma (American type) (22), there has been a controversy over the species of origin of this virus, now designated ESP-1 virus. It has been suggested by Gilden et al. (14) that the ESP-1 virus contains the groupspecific (gs-1) antigen characteristic of MuLV's. However, these studies utilized ESP-1 cell homogenates and only weak reactions were observed. More recent immune diffusion studies utilizing concentrated ESP-1 virions disrupted by Tween 80:ether have shown that the ESP-1 virus particles do not contain either the murine gs-1 or feline gs-1 antigen (6). In contrast, murine gs-1 was readily detected in type C virus particles from HEK-RLV (3).

Following the observation by Gallo et al. (13) that ESP-1 virus contains an RNA-dependent DNA polymerase or "reverse transcriptase" (13), it was demonstrated by Scolnick et al. (24, 25) that this enzyme could be inhibited by sera capable of inhibiting the reverse transcriptase of MuLV and FeLV. These results indicated that the ESP-1 polymerase may be immunologically related to both the murine and feline enzymes, but the results do not necessarily represent species-specific reactions. On the contrary, Todaro and Gallo (29) have recently demonstrated that reverse transcriptase isolated and purified from fresh (not cultured) human leukemia cells is related to the murine and/or primate viral reverse transcriptase. Reverse transcriptase from some human leukemic cells has both the biochemical and antigenic properties of reverse transcriptase from both murine and primate type C virus. Dalton (7) demonstrated that ESP-1 virus particles differ morphologically in the thickness and position of the intermediate layer from type C particles of both MoLV and HEK-RLV.

In an attempt to characterize further the ESP-1 virus, experiments were carried out to determine whether an in vivo helper assay (26) could be used to quantify and subsequently to determine the type-specific envelope antigenicity of ESP-1 virus.

MATERIALS AND METHODS

Animals. Male and female 6- to 8-week-old C57BL/6, DBA/2Ha, BALB/cCr, and C3H/He mice were obtained from the West Seneca Animal Production Unit, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, N. Y. 14203.

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Memorial Institute, Buffalo, N.Y. In addition, female rats were obtained through the courtesy of Samuel M. Poiley, National Cancer Institute, Bethesda, Md.

Viruses. Defective SFFV, as contained in the Mirand strain (19) of the FV complex, has now undergone over 200 cell-free passages in Ha/ICR Swiss mice. All such stocks initiate infection 50 to 100 times more efficiently in Swiss and DBA/2 mice than in BALB/c mice (8) and are therefore designated as having an N-tropic host range (15).

A B-tropic pseudotype of Friend SFFV was prepared using a leukemia-inducing helper virus isolated by Tennant (8). This pseudotype is referred to here as SFFV(TenLV) and is defective for spleen focus formation in N-type mice (18.20). Because of this strict host range, it was possible to design and execute helper virus assays in N-type mice using SFFV-(TenLV) as the defective “indicator” virus. In addition, routine helper assays were conducted in B-type mice (BALB/c) using FV complex (containing SFFV and N-tropic LLV-F) as indicator. The isolation of LLV-R from the murine RV complex [SFFV(LLV-R)] has been described previously (27). LLV-R stocks were maintained by serial passage of 20% (w/v) cell-free extracts of leukemic tissue in newborn BALB/c mice and were stored at -196°. All LLV-R preparations express significant levels of helper activity in both N- and B-type mice and are able to induce XC plaques in NIH Swiss and BALB/c mouse embryo fibroblasts with equal efficiency. LLV-R is therefore designated as having an NB-tropic host range (15).

ESP-1 virus was prepared from fluids of cultures of ESP-1 cells grown in minimal essential medium with N-tris(hydroxymethyl)methylglycine. The virus was partially concentrated by the use of the Amicon Diaflow apparatus and further centrifuged at 22,500 rpm (46,000 X g) in a Spinco No. 40 rotor for 2.5 hrs. The pellet was resuspended in PBS to equal a 200-fold concentration.

Rauscher leukemia virus adapted to human embryo kidney cells (HEK-RLV) was obtained from fluids from tissue cultures of the cell line, HEK-RLV. Preparation of the virus concentrate was identical to that of ESP-1 virus. The HEK-RLV cell line was obtained originally from the John F. Smith Memorial Laboratory of Pfizer Pharmaceutical Co., Maywood, N.J., and maintained in our laboratories.

ESP-1-derived Mycoplasma was received from Dr. J. Fogh, Walker Laboratories, Rye, N.Y. The mycoplasmas were isolated from the ESP-1 cells in broth culture and passage 10 times. Subsequently, human amnion cells (FL) were inoculated with Mycoplasma and tissue culture fluids were then concentrated in a manner similar to that of the ESP-1 and HEK-RLV viruses. This provided us with a large stock of ESP-1 derived Mycoplasma for these studies.

Virus Titrations. All SFFV preparations were titrated in vivo with the spleen focus assay method (4). Briefly, 0.5-ml samples of diluted SFFV were injected i.v. into susceptible mice. After 9 days their spleens were removed and fixed in Bouin’s solution. Discrete foci on the splenic surface were counted macroscopically, and virus titers (mean number of foci per spleen X dilution factor) were expressed in FFU/ml, where 1 FFU is that amount of virus required to induce an average of 1 focus per spleen. All LLV-R, ESP-1 virus, and HEK-RLV stocks were free of detectable SFFV and demonstrated helper activity for SFFV.

The quantification of MuLV’s with a helper virus assay method has been described (28). Briefly, a constant amount of FV complex containing helper-dependent “indicator” SFFV was added either to potential helper virus, serially diluted from 1:10 to 1:1000 in PBS, or to PBS alone. Samples of 0.5 ml were then injected into the lateral tail vein of male BALB/c mice, 7/group. After 9 days, the mice were killed, their spleens were fixed in Bouin’s fluid, and the number of discrete foci on the splenic surface that were >0.5 mm in diameter were counted macroscopically. The mean number of foci per spleen for each group, when multiplied by the SFFV dilution factor, gave an estimate of the titer of SFFV with or without diluted helper virus. The difference between these 2 estimates (i.e., the helper virus activity in Δ FFU/ml) was then related on a log/log plot to the dilution factor of the helper virus. The linear extrapolation for the helper activity of undiluted virus is expressed in HU, where 1 HU is that amount of virus required to increase the estimated titer of SFFV by 1 FFU/ml.

Preparation of Murine and Rat Antiserum. Rauscher antiserum was prepared in adult C57BL/6 mice given 4 biweekly i.p. injections of 10⁸ freeze-thawed BALB/c leukemic cells induced by RV complex, SFFV(LLV-R). The 1st injection included 0.02 ml of complete Freund’s adjuvant. Moloney antiserum was prepared in adult BALB/c mice given 20 weekly i.p. injections of 10⁸ freeze-thawed BALB/c lymphoma cells induced by MoLV. The 1st immunization also included complete Freund’s adjuvant as described above. Within each series of immunizations, the mice were bled 7 days after the last injection. Sera were then pooled, heated at 56° for 30 min to eliminate complement activity, and stored at -70°. Rauscher antiserum was serially adsorbed 3 times with “normal” BALB/c spleen cells to remove alloantibodies that have previously been shown to be present in sufficient quantities to neutralize Rauscher and Friend SFFV. All sera demonstrated virus-neutralizing activity against the MuLV corresponding to the MuLV-induced leukemia cells used as pretreatment materials. These activities were completely removed by 3 adsorptions with appropriate MuLV-induced leukemia cell homogenates. As a control, NMS was obtained from an untreated group of BALB/c mice.

In an attempt to prepare specific neutralizing antiserum directed against ESP-1 virus, New Zealand Black rats were immunized with Tween:ether-treated ESP-1 virus purified 2 times on sucrose cushions. The 1st injection (1.0 ml s.c.) included 0.5 ml of complete Freund’s adjuvant. The 2nd and 3rd injections (1.0 ml s.c.), 0.5 ml of which was Freund’s incomplete adjuvant, were given on Days 7 and 14, respectively. The 4th injection was given on Day 21 i.p. Seven days after the last treatment, the animals were bled.

Helper Virus Neutralization. The antigenic properties of LLV-R, HEK-RLV, and ESP-1 virus were compared in terms of the neutralization of their helper activity when incubated for 30 min at 37° with either specific murine antisera or antiserum prepared from rats immunized with ESP-1 virus and antiserum prepared against Mycoplasma from ESP-1. Helper virus (final dilution, 1:10) was added at time zero to 0.2 ml of antiserum or NMS and 0.8 ml of PBS, which had been prewarmed in a 37° water bath. After 30 min, the entire sample was added to 0.1 ml of ESP-1 virus and an enzyme-linked immunosorbent assay was used to detect helper activity.

* R. J. Eckner, unpublished data.
RESULTS

ESP-1-derived Helper Activity for Defective Friend SFFV

Recent experiments have shown that the expressed helper dependence of SFFV for focus formation is in fact a reflection of SFFV dependence upon its associated helper virus (LLV-F) for late stages in virus synthesis (8). MuLV helper is required for the replication of infectious SFFV since SFFV acquires the type-specific envelope of its associated helper with subsequent release from infected cells. Spleen foci develop rapidly via spread of SFFV infection, while a cell infected by SFFV only, and transformed, forms a focus more slowly and may not be initially detected since the focus must enlarge by cell division alone. A rapid in vivo assay (28) for detecting MuLV helper activity is based on this expressed defectiveness of SFFV and focus formation. This helper assay has been successfully used to detect and quantitate 10 different strains of MuLV (28), as well as FeLV and hamster leukemia virus. Since this assay can apparently be used to detect type C viruses across species barriers, experiments were carried out to determine whether the ESP-1 virus (presumably of human origin) could provide a similar helper function. Accordingly, N-tropic FV complex containing helper-dependent "indicator" SFFV was added to either a highly concentrated ESP-1 virus preparation (see “Materials and Methods”), serially diluted from 1:32 to 1:2048 in PBS, or to PBS alone. All virus preparations were immediately assayed in BALB/c mice. As shown in Chart 1, this virus preparation expressed extremely high levels of apparent helper activity for defective SFFV, and the slope of the regression line was typical of those obtained using active MuLV helper preparations. To date, 4 different ESP-1 virus preparations have been tested and have been shown to be active helpers. Titer estimates have ranged from 10^4 to >10^5 HU/ml. This same ESP-1 virus preparation that provided significant levels of helper activity for SFFV when assayed in BALB/c mice (Chart 1) was also assayed in DBA/2 mice (Fv-I^nn) using the B-tropic SFFV(TenLV) as a defective indicator (8). Two different preparations of Rauscher virus (HEK-RLV and an NB-tropic LLV-R helper) were also tested and were shown to be positive in such tests, while ESP-1 virus failed to express a helper function when assayed in these N-type mice (Table 1). It was an unexpected finding for a virus grown in human cells to express a strict B-tropic host range in the murine system. However, the observation of a strict host range marker associated with ESP-1-derived helper activity suggests that this activity is not attributable either to HEK-RLV contamination of the ESP-1 cell line (since LLV-R and HEK-RLV are NB-tropic) or to nonspecific interactions between SFFV and ESP-1 preparations within a common host. Immunosuppressive agents or factors able to increase the number of available target cells for SFFV would be expected to mimic a helper effect in all strains of mice (9, 10). However, because ESP-1-derived helper preparations contain both type C virus and Mycoplasma-like agents, it was important to determine the specificity of this helper activity and the role of the ESP-1-derived Mycoplasma (if any) in our system. These matters are considered in detail in the following 2 sections.

Association of ESP-1-derived Helper Activity with the Presence of Type C Virus. From the experiments described, it can be seen that culture fluids from the ESP-1 cell line contribute an apparent helper function to SFFV. However, these fluids contain both type C virus and Mycoplasma-like organisms. It has been demonstrated previously (10) that Mycoplasma-like organisms may enhance the estimated titer of defective SFFV and thus mimic real helpers. Fortunately, however, there are methods available to determine whether the observed "helper activity" is real and directly associated with the presence of type C virus particles. The extremely high level (>10^5 HU) of helper activity expressed by concentrated ESP-1 culture fluids made it possible to utilize a sucrose gradient analysis and determine whether this activity was directly associated with the presence of ESP-1 virus. Partially purified material, 22 ml from 4 liters of tissue culture fluids of ESP-1 culture, was layered onto a 33-ml gradient of 1.13 to 1.25 sucrose per ml prepared in a buffer of 0.1 M NaCl:0.001 M EDTA:0.01 M Tris-HCl, pH 7.6. The material was centrifuged

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<th>HELPER ACTIVITY (A. FU/ml)</th>
<th>DILUTION FACTOR OF ESP-1 HELPER</th>
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<tr>
<td>10^4</td>
<td>1:10</td>
</tr>
<tr>
<td>10^3</td>
<td>1:100</td>
</tr>
<tr>
<td>10^2</td>
<td>1:1,000</td>
</tr>
<tr>
<td>10^1</td>
<td>1:10,000</td>
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<tr>
<td>10^0</td>
<td>1:100,000</td>
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Chart 1. Relationship between helper activity (mean ± S.E.) and the dilution factor of ESP-1 virus added to a stock of defective SFFV(LLV-F) indicator. The slope of the regression line is −0.77, and the y intercept is 16.4 × 10^4 HU.

ESP-1 Virus

**Table 1**

<table>
<thead>
<tr>
<th>Helper preparation</th>
<th>Host (genotype)</th>
<th>Helper activity (HU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESP-1 virus</td>
<td>BALB/c (Fv-I^bb)</td>
<td>164,000 ± 15,000</td>
</tr>
<tr>
<td></td>
<td>DBA/2 (Fv-I^nn)</td>
<td>0</td>
</tr>
<tr>
<td>HEK-RLV</td>
<td>BALB/c</td>
<td>95,000 ± 29,000</td>
</tr>
<tr>
<td></td>
<td>DBA/2</td>
<td>45,000 ± 8,000</td>
</tr>
<tr>
<td>LLV-R</td>
<td>BALB/c</td>
<td>16,100 ± 2,400</td>
</tr>
<tr>
<td></td>
<td>DBA/2</td>
<td>24,000 ± 3,300</td>
</tr>
</tbody>
</table>

a All virus stocks were prepared as described in “Materials and Methods.”

b Each helper virus assay utilized a minimum of 4 dilutions of candidate helper virus and 7 mice/group.

c Mean ± S.E.
for 24 hr at 22,500 rpm (73,000 X g) in a Spinco No. 25.2 rotor.

The gradient was fractionated, and the 7 fractions between 1.07 and 1.22 g/ml were diluted with 0.1 M NaCl:0.001 M EDTA:0.01 M Tris-HCl buffer, pH 7.4, and then centrifuged for 2 hr at 22,500 rpm (46,000 X g) in a Spinco No. 40 rotor. The resulting pellets were resuspended in 4 ml PBS.

Aliquots (1000-fold concentrated) were assayed for helper activity in BALB/c mice. For estimation of presence of virus particles, aliquots of 0.6 ml of each density gradient fraction were centrifuged onto Millipore membranes at 25,000 X g in an SW 39 rotor for 1 hr. The materials on the membranes were fixed with glutaraldehyde and osmic acid according to the procedure described by Miller et al. (18) and examined by thin sectioning.

The results shown in Chart 2 indicate that significant levels of helper activity could be recovered from gradient samples ranging in density from 1.15 to 1.17 g/ml, and maximum activity was recovered from the sample of density of 1.16 g/ml. All 3 samples contained ESP-1 virus and Mycoplasma (Figs. 1, 2a, and 2b). However, other samples containing predominantly Mycoplasma-like organisms and a very rare virus particle failed to show a helper effect (Chart 2). This suggests that the Mycoplasma organisms alone do not act as helpers. In addition, it has been demonstrated that “pure” ESP-1-derived mycoplasmas grown in broth do not exert a synergistic effect when added to a known MuLV helper preparation. The titer estimate for an active NB-tropic LLV-F helper stock was observed to be 10.3 X 10^3 HU/ml. In the presence of a high concentration of excess ESP-1-derived Mycoplasma, the titer estimate for this same LLV-F preparation was 9.9 X 10^3 HU/ml. In addition, the slope of the dose-response curve for LLV-F helper activity was not altered (slope = -0.186 and -0.19, respectively).

Conversion of SFFV Dose-Response Titration Patterns to a near-1-Hit Form in the Presence of Excess ESP-1 Helper Virus. The Fv-1<sup>b</sup> gene of B-type mice (20, 21) interferes with MuLV with an N-tropic host range. Thus, infection of adult BALB/c mice (Fv-1<sup>b<sub>b</sub></sup>) with several different dilutions of FV complex containing SFFV and N-tropic LLV-F helper yields a multiple-hit dose-response curve (8, 11). Addition of excess MuLV helper converts the titration pattern to a 1-hit or near-1-hit form indicative of the expression of true helper activity for defective SFFV (Chart 3; Refs. 8 and 10). In contrast, recent experiments have shown that enhancement of SFFV infectivity in BALB/c mice by Mycoplasma (9, 10) and immunosuppressive agents (11) is associated with a shift of the dose-response curve upward in the absence of conversion of the multiple- or 2-hit curve to a 1-hit pattern. Because some ESP-1-derived Mycoplasma stocks did express apparent helper activity for SFFV in both N- and B-type mice, this type of analysis was used to determine whether this activity represented real activity or enhancement. Accordingly, a preparation of FV complex containing SFFV and N-tropic LLV-F was serially diluted into PBS alone or into either an active preparation of ESP-1 virus (final dilution, 1:20) or an active ESP-1-derived Mycoplasma (final dilution, 1:10). All SFFV preparations were immediately assayed in BALB/c mice, 7/group. The results (Chart 3) demonstrate a striking increase in the number of spleen foci observed when SFFV was diluted into the ESP-1 virus preparation. The increased number of foci near the end point was sufficient to convert the slope of the dose-response curve from a multiple-hit to a near-1-hit form. In contrast, the dose-response pattern for SFFV titrated in the presence of excess ESP-1-derived mycoplasma remained multiple-hit. This indicates that SFFV is highly defective in the presence of excess Mycoplasma and that such materials enhance rather than help SFFV. These data provide further evidence that the activity provided by ESP-1 virus is real helper activity and not solely attributable to the presence of Mycoplasma. Also, mycoplasmas are able to enhance the expression of Friend SFFV in several strains of N- and B-type mice.
were undergoing mitotic division. of Friend disease (Fig. 3). Foci appeared as clusters of morphology of SFFV-induced spleen foci produced in the presence of excess ESP-1 helper virus were indistinguishable from those induced by SFFV(LLV-F) alone and were typical of Friend disease (Fig. 3). Foci appeared as clusters of immature erythroid tumor cells. More than 80% of these cells were undergoing mitotic division.

Analysis of the Type-specific Envelope Antigen(s) of ESP-1 Helper Virus

It has been shown in a previous study (27) that incubation of MuLV helper virus with type-specific murine antiserum neutralizes the helper activity associated with these viruses. Since ESP-1 virus and HEK-RLV both provide a helper function to SFFV, it was possible to compare the type-specific envelope antigenicity of ESP-1 helper virus to that of HEK-RLV, as well as to our own LLV-R stocks. This study was carried out to investigate the contention that the virus designated ESP-1 may represent an HEK-RLV contaminant of the ESP-1 cell line. All virus preparations were incubated with antiserum directed against Rauscher virus, Moloney antiserum, and rat antiserum prepared against ESP-1 virus (see "Materials and Methods"). After 30 min of incubation at 37°, each reaction mixture was diluted in ice-cold 0.9% NaCl solution and spun at 100,000 X g for 90 min. The resulting pellets were resuspended in 1.0 ml of 0.9% NaCl solution and assayed for helper activity for defective SFFV in BALB/c mice. The results of these experiments are shown in Table 2. Helper activity is expressed in Δ FFU/ml rather than HU/ml because large quantities of both virus and serum are necessary to quantitate virus (incubated with and without antiserum) in HU/ml. Because the calculated slope of the regression line for LLV-R, ESP-1, and HEK-RLV helper activity was reasonably constant and the HU values derived using these regression lines did not change significantly from one experiment to another, it was possible to use effectively both helper viruses and all neutralizing antiserum at standard doses. As a control, an aliquot of ESP-1 virus and HEK-RLV was incubated with NMS and assayed for helper activity before and after centrifugation. As shown in Table 2, some virus activity was lost following the centrifugation procedure. However, the levels of activity observed were large enough to allow a comparison with the values obtained utilizing specific antiserum. It can be seen that both Rauscher and Moloney antiserum neutralized ESP-1 helper virus activity but that they did not neutralize LLV-R and HEK-RLV. In contrast, ESP-1 antiserum neutralized the ESP-1 virus activity but failed to neutralize LLV-R and HEK-RLV. The neutralizing activity of ESP-1 was adsorbed using ESP-1 virus, but Moloney antisera failed to neutralize ESP-1 virus activity. These data indicate that, using 3 different type-specific antiserum, we can detect virus-bound, envelope antigen(s) on ESP-1 virus that are distinct from those neutralizable antigens present on both LLV-R and HEK-RLV. Using this system, neutralizable antigens could be detected common to Rauscher virus grown both in mice and in human cells in vitro. This is consistent with the observation of Aaronson (1) and does not rule out the possibility that the human-grown virus has at least some modified surface antigens not detected using these antiseras.

DISCUSSION

The RNA-containing tumor viruses are now recognized as being complex with respect to both structure and function. An analysis of the defectiveness of Friend SFFV has not only increased our understanding of the relationship between SFFV and the MuLV helper viruses but has also shown that this defective virus may be exploited to detect and characterize any virus isolate that provides a helper function to defective SFFV. The present study is specifically concerned with the type C virus isolate designated ESP-1. Since the 1st observation of this virus in a monolayer cell culture originally established from human tissue (22), it has been speculated by some that ESP-1 virus is an endogenous human type C virus. In contrast, others have suggested that ESP-1 virus is the end result of Rauscher
virus contamination of the ESP-1 cell line. To shed some light on this controversy, ESP-1 virus was tested for its ability to provide a helper function to defective SFFV and subsequently compared with HEK-RLV. The ESP-1 virus does in fact provide a true helper function for defective SFFV (Charts 1 to 3), and the expression of this activity is very similar to that provided by the MuLV's. ESP-1 virus appears to be B-tropic in that helper activity could be detected in BALB/c mice (Fv-lbb) but not in DBA/2 mice (Fv-lnn) (Table 1). In contrast, HEK-RLV is NB-tropic with respect to its ability to provide a helper function to SFFV. An NB-tropic host range marker is usually stable within the murine system4, and this suggests that ESP-1 virus is not an HEK-RLV contaminant. It has been shown previously (1) that the host range of RLV may change after growth in human cells. However, this observation may have resulted from the selection of a xenotropic virus (17) present in RLV stocks. Such viruses have now been isolated from several species and it is possible that we may have detected (in the present study) an endogenous ESP-1 cell-derived virus with a specifically xenotropic host range. The possibility still exists, however, that ESP-1 virus is a distinct B-tropic variant of RLV, but the host range conversion of RLV after extensive growth in human cells is usually species-specific (xenotropic) and associated with expansion of infectivity to one host (human) at the expense of another host (murine) (1–3).

Because ESP-1 virus stocks provided easily detected levels of helper activity for SFFV, it was possible to determine the basic antigenic composition of the type-specific virus envelope. Type-specific murine antisera prepared against RLV and MolLV known to cross-react strongly with RLV (12) neutralized the expressed helper activity of HEK-RLV and LLV-R but did not neutralize ESP-1 virus-derived helper activity. A reciprocal pattern of neutralization was seen using rat antiserum prepared against ESP-1 virus. These data indicate that ESP-1 virus possesses neutralizable envelope antigens not present in HEK-RLV.

This antigenic analysis of ESP-1 virus is not as quantitative as a previous analysis of MuLV type-specific envelope antigens utilizing the technique of virus neutralization kinetics in vitro (12). However, to date we have not been able to prepare an ESP-1 pseudotype of SFFV and such a study must await the results of this pseudotype. ESP-1 is a distinct virus but the results obtained in the present study do not totally rule out the possibility that ESP-1 virus is a stable, antigenically distinct variant of RLV. This possibility is remote since, as shown in the present study (Table 2) and by others (1, 3, 28), RLV grown in human cells retains some murine-grown RLV type-specific antigen(s). An alternative explanation for the observed patterns of neutralization is that HEK-RLV grown in the ESP-1 cell line undergoes a unique antigenic change due specifically to an RLV-ESP-1 interaction. To test this possibility we have attempted intentionally to infect ESP-1 cells with HEK-RLV. To date, however, we have not detected the expression of the MuLV group-specific (gs-1) antigen in these cells or an increase in the number of virus particles produced and assume therefore that these cells are not yet infected. These experiments are continuing.

Finally, the control of gs antigen synthesis by defective and competent MuLV should be considered briefly. Species-specific gs antigenic markers are not usually found in detectable quantities in cells harboring a defective yet rescuable viral genome. An exception to this pattern of gs expression has been reported by Sarma et al. (23) utilizing the Gazdar strain of murine sarcoma virus. A rescued murine sarcoma virus carries the gs-1 antigenic marker of the species of origin of the helper virus used. How do these facts relate to ESP-1 virus? No murine or feline gs-1 antigen can be detected in ESP-1 virions by immunodiffusion. In contrast, RLV grown in human cells retains the murine gs-1 antigen (30). On the basis of these observations, it can be assumed that ESP-1 virus is not RLV. Also, a more conservative model can be proposed that is consistent with the detection of human-derived type C virus markers. RV complex contains a defective focus-forming virus (SFFV) and LLV-R helper (12). If defective SFFV alone had infected the ESP-1 cell line, murine gs-1 antigen would not be detected. However, interaction between this exogenous viral genome and endogenous human viral information could result in the expression of a complete, infectious type C virus. The resulting "hybrid" virus would carry the RLV genome, an ESP-1 virus-derived envelope, and a "human" ESP-1-derived gs antigen(s); it would perhaps be associated with complete ESP-1 helper virus. This proposed model is feasible since we know that both Friend and Rauscher SFFV's productively infect fibroblastic cells in vitro (R. J. Eckner and N. Paolini manuscript in preparation). Further a 70 S RNA-instructed DNA polymerase has been detected in 87% of Burkitt's lymphomas tested (16, 26). This proposed model is testable and can be critically appraised as our knowledge of the RNA tumor viruses, their interaction with host cells, and more virus isolates become available for experimentation. It should remain as a possible mechanism responsible for the detectable expression of viral information in human cells.

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Fig. 1. Electron micrograph of a crude concentrate of ESP-1 tissue culture fluid. Immature and mature virus particles (single arrow) may be seen. Mycoplasma, including elementary bodies, is also present (double arrows). X 45,000.

Fig. 2. Electron micrographs showing ESP-1 virus particles in the 1.16 to 1.17 g/ml sucrose gradient sample. X 45,000.

Fig. 3. a, gross appearance of the spleens of BALB/c mice infected 9 days earlier with either N-tropic FV complex alone (left; average number of foci per spleen = 2.93) or the same dose of FV complex in the presence of excess ESP-1 virus (right; average number of foci per spleen = 78.9). b, histological section prepared from the spleen of a BALB/c mouse inoculated with both FV complex and ESP-1 virus. SFFV-induced foci appear as clusters of immature erythroid cells, most of which are seen as mitotic figures. H & E, x 400.
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