Biological and Antigenic Characteristics of HEL-12 Virus

By CAROLYN M. BERGHOLZ, JOHN T. REYNOLDS, AND SANDRA PANEM

Department of Microbiology and School of Basic Medical Sciences, University of Illinois, Urbana, Illinois, U.S.A. and Departments of Microbiology, Pathology, and the Committee on Virology, University of Chicago, Chicago, Illinois, U.S.A.

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SUMMARY

The biological and antigenic properties of HEL-12 virus have been compared with gibbon ape lymphosarcoma virus (GALV) and simian sarcoma and simian sarcoma-associated viruses, SiSV and SSAV, respectively. HEL-12 virus did not transform human or marmoset fibroblasts but rescued SiSV focus-forming activity from non-productively transformed marmoset cells (HF/SiSV-NP). Like SSAV and GALV, HEL-12 virus induced syncytia with XC cells. In addition, HEL-12 cells which did not produce virus but which contained HEL-12 proviral DNA, rescued SiSV from HF/SiSV-NP cells in co-cultivation experiments. Results of neutralization and serum cytotoxicity tests utilizing SiSV rescued by HEL-12 [SiSV-(HEL-12)] indicated that HEL-12 virus envelope proteins are very closely related to those of SSAV but readily distinguished from those of GALV. Antigenic diversity of SiSV(SSAV), SiSV(GALV) and SiSV(HEL-12) envelope glycoproteins (gp70) was shown in competition radioimmunoassays (RIA) designed to detect minor antigenic differences using antiserum monospecific for SiSV(SSAV) gp70 or Friend murine leukaemia virus gp70. Antigenic differences between these gp70s were demonstrated in RIA using purified SSAV gp70 or HEL-12 gp70. These data indicate that HEL-12 virus has biological properties similar to those of SSAV and GALV, is distinguished from GALV in neutralization tests and has both distinct and SSAV-related gp70 antigenic determinants.

INTRODUCTION

Although an endogenous C-type virus has not yet been isolated from human tissue, the evidence for C-type virus expression in man continues to occur. Serological studies indicate that humoral and cell-mediated immunological responses to antigens which share cross-reactive determinants with those of the woolly monkey fibrosarcoma/gibbon ape lymphosarcoma (SiSV/GALV) group of exogenous primate viruses, as well as the baboon endogenous viruses (BaEV), are widespread in the human population (Prochownik & Kirsten, 1976; Snyder et al. 1976; Kurth et al. 1977; Hirsch et al. 1978; Jacquemin et al. 1978; Panem et al. 1978; Thiry et al. 1978).

The isolation and preliminary characterization of a C-type virus spontaneously released by a strain of normal human embryonic lung fibroblasts HEL-12 has been reported (Panem et al. 1975). Previous studies have established that although some human tissues contain

* To whom reprint requests should be addressed.
proviral sequences which are homologous to the HEL-12 virus genome, HEL-12 virus is not an endogenous virus of man (Prochownik & Kirsten, 1977). Nevertheless, surveys of human sera for antiviral antibodies (Prochownik & Kirsten, 1976) and of human tissue for virus antigen (Panem et al. 1978; Sawyer et al. 1978) indicate that HEL-12 virus-related antigens are detectable in man.

It was previously reported that HEL-12 virus had a p30 structural polypeptide and a reverse transcriptase which are immunologically related to those of the SiSV/GALV virus group and that HEL-12 was infectious for a broad range of mammalian cells (Panem et al. 1975, 1977). In view of these findings it was of interest to compare further the biological and antigenic properties of HEL-12 virus with transforming and non-transforming isolates of the SiSV/GALV group of exogenous primate viruses.

METHODS

Cells. SiSV-transformed non-producer marmoset clonal cell lines, HF/SiSV-NP-I, V and VI, have been described (Bergholz et al. 1977a). Cells producing primate retroviruses were human embryonic lung cell strain 12 (HEL-12) at passages 27 to 32 (Panem et al. 1977); SiSV-transformed marmoset fibroblast cells producing SiSV and a 10- to 100-fold excess of SSAV [HF/SiSV(SSAV)]; HF/SiSV-NP superinfected with HEL-12 virus or GALV-San Francisco strain and producing the respective pseudotypes of SiSV, SiSV-(HEL-12) or SiSV(GALV).

Normal cells included human foreskin fibroblasts (HFF) and marmoset foetal skin cells (MFS), established in culture in the Department of Microbiology at Rush Presbyterian-St. Luke’s Medical Center, Chicago, Ill., U.S.A. and human embryonic lung cell strain 8 (HEL-8). HEL-8 cells were the gift of Drs M. O. Beem and E. Saxon, Department of Pediatrics, University of Chicago, Chicago, Ill., U.S.A. Non-virus-producing HEL-12 cells at passage 6 (HEL-12 p6) (Panem et al. 1977) and XC rat cells carrying the Rous sarcoma virus genome (Klement et al. 1969) were also employed. Conditions for the growth of each cell strain have been described previously (Bergholz et al. 1977a, b; Panem et al. 1977).

Viruses. Cell-free virus for infectivity, serum neutralization, rescue and reverse transcriptase assays were obtained as spent culture fluids of virus-producing cell cultures described above. Twice gradient-purified viruses were used for competition radioimmunoassay. Simian sarcoma–simian-associated virus [SiSV(SSAV)] grown in 71-AP-1 marmoset cells was from Electronucleonics Laboratories, Bethesda, Md., U.S.A. GALV grown in NC37 human cells was obtained from Pfizer, Inc., Maywood, N.J., U.S.A. through the auspices of the Biological Carcinogenesis Branch, National Cancer Institute, Bethesda, Md., U.S.A. SiSV(HEL-12) virus was purified by isopycnic centrifugation as previously described (Panem et al. 1975).

Virus assays. For assay of focus-forming virus, MFS or HFF cells were infected as described previously (Bergholz et al. 1977a). Mixed culture cytopathogenicity tests with XC cells were performed according to the method of Klement et al. (1969). Neutralization was determined by inhibition of focus induction following reaction of 100 focus-forming units (f.f.u.) of virus with twofold dilutions of antiserum for 4 h at 4 °C (Bergholz et al. 1977b). Surviving fraction (Vs/Vo) was calculated relative to virus (Vo) incubated in parallel in the absence of antiserum. Complement-dependent serum cytotoxicity was measured in a 51Cr-release microassay with neutralizing goat anti-SSAV serum (Bergholz et al. 1977a). Percent specific cytotoxicity was calculated as

\[
\frac{\text{ct/min test release} - \text{ct/min control release}}{\text{ct/min maximum release} - \text{ct/min control release}} \times 100
\]
Values for control release included the spontaneous release, cells incubated with antibody alone, or with complement alone. The highest control release value was used for calculating percent specific cytotoxicity. Tests were performed in quadruplicate and standard deviations were calculated.

For assay of helper virus activity and production of SiSV pseudotypes, HF/SiSV-NP cells were either superinfected with cell-free virus or co-cultivated with virus-producing cells (Bergholz et al. 1977a). Cells were subcultured after 1 week. Spent culture fluids were collected, clarified by centrifugation and assayed for focus-forming activity on HFF or MFS cells. Supernatants from uninfected HF/SiSV-NP cells and helper virus-producing cells cultured alone were tested in parallel.

**Antisera.** Neutralizing goat anti-SiSV(SSAV) serum was prepared by hyperimmunization of a goat with autologous SiSV-transformed cells (Deinhardt et al. 1978). Polyacrylamide gel electrophoresis (PAGE) of immunoprecipitates of labelled SiSV(SSAV) demonstrated reactivity primarily and almost exclusively against the envelope components of SiSV(SSAV), i.e. gp70, p15(E)/p12(E), although some activity against p30 was detected. Rabbit antisera specific for the gp70 of SiSV/SSAV, prepared in collaboration with Drs H. J. Thiel, W. Schäfer and F. Deinhardt, has been characterized (Thiel et al. 1978). Antiserum, specific for gp70 of Friend murine leukaemia virus (FLV), was also prepared by Dr Schäfer and co-workers (Schäfer et al. 1975) and kindly provided by Dr R. Kurth (Max Planck Institute, Tübingen, West Germany). Anti-SiSV(SSAV) was raised by hyperimmunization of a rabbit with NP-40-disrupted virus grown in 71-AP-1 cells (Panem et al. 1976). The antisera has been characterized by immunoprecipitation followed by PAGE and shown to react with the virus gp70, p30 and p15(E). Antisera were stored in aliquots at −20 °C or −70 °C and inactivated at 56 °C for 30 min before use. Normal rabbit serum stored in aliquots at −70 °C served as a source of complement for cytotoxicity tests.

**Sodium dodecyl sulphate (SDS)–PAGE.** Samples for PAGE were solubilized in 2 % SDS, 5 % 2-β-mercaptoethanol, 0.05 M-tris, pH 7, 0.005 % bromophenol blue and 1 % sucrose at 100 °C for 2 min. Samples were layered on to analytical 8.5 cm, 10 % polyacrylamide slab gels with 3 % stacking gels (Laemmli, 1970). The cross-linking agent was NN'–diallyltartardiamide. Gels were run with tris-glycine buffer, pH 8.0, at constant current (20 mA/gel) until the bromophenol blue dye migrated within 1 cm of the bottom of the gel. Gels were stained overnight with 0.025 % Coomassie brilliant blue, 10 % acetic acid and 25 % isopropanol, destained in 10 % acetic acid alone (Fairbanks et al. 1971) and were dried on to 3MM filter paper (Whatman, Maidstone, U.K.). Autoradiograms were made with Kodak SB X-ray film (Eastman-Kodak, Rochester, N.Y., U.S.A.). The apparent mol. wt. of proteins were calculated using the procedure of Shapiro et al. (1967) with albumin (68 000 mol. wt.), ovalbumin (45 000 mol. wt.), chymotrypsinogen (25 000 mol. wt.) and ribonuclease A (13 700 mol. wt.) (Pharmacia, Uppsala, Sweden) as standards.

**Preparation of iodinated virus antigens.** Disrupted, gradient-purified virus was labelled with $^{125}$I as described by Hunter (1967) and modified by Montelaro & Rueckert (1975) to a specific activity of approx. 10$^6$ ct/min/µg virus protein. Virions were disrupted by incubation at 37 °C for 30 min in 0.1 M-potassium phosphate-buffered saline, pH 7.5 (PBS) containing 1 % NP-40, followed by sonication. $^{125}$I (1 to 2 mCi) in NaOH (New England Nuclear Corp., Boston, Mass., U.S.A.) was added to 500 µg disrupted virus in the presence of 50 µM-KI. All reagents were prepared in PBS and the reaction was carried out at 4 °C. Chloramine T (0.4 µg/µg virus protein) was added to initiate the reaction and after 2 min the reaction was stopped by addition of an equimolar amount of sodium metabisulphite. Free iodine was separated from labelled protein by chromatography over Sephadex G25 (fine; Pharmacia) equilibrated in PBS. Labelled protein was dialysed extensively against PBS.
Iodinated virions were solubilized with SDS and sulphydryl reagents and the individual virion proteins were separated by PAGE (Colcher et al. 1978). Following electrophoresis, the labelled gp70s were located by slicing a lane of the slab gel into 1 mm sections and determining radioactivity in a gamma spectrophotometer. Protein was eluted from the appropriate gel slices containing gp70 by incubation at 37 °C for 2 h in PBS containing 0.5% NP-40. The gp70 antigens prepared by this method were 80% precipitable by monospecific rabbit antiserum prepared to gp70. Pre-immune serum did not precipitate gel-fractionated antigens. Less than 5% of SSAV gp70 was precipitated by anti-SiSV(SSAV) p30 serum. Gel-eluted antigens were re-electrophoresed on SDS–PAGE and more than 90% of the radioactivity migrated as a single band. Gel-purified gp70s were stored at -70 °C and used in competition radioimmunoassay. Protein concentrations were determined by the method of Lowry et al. (1951).

Radioimmunoassay. All radioimmunoassay determinations were done in duplicate. For competition radioimmunoassay, unlabelled competing antigen was incubated at 37 °C for 1 h with limiting dilution of antibody previously determined to precipitate 50% of the labelled antigen. Approx. 5000 to 10000 ct/min 125I-SiSV(SSAV) p30 or SiSV(SSAV) gp70 were then added and the incubation continued for 1 h at 37 °C. Reactions were done in a final vol. of 200 μl in PBS containing 0.5 mg/ml bovine serum albumin, and 0.1% NP-40 (PBA buffer). Formalin-fixed Staphylococcus aureus, Cowan strain I [50 μl of a 10% (v/v) solution], was then added and allowed to bind the antigen-antibody complexes for 30 min at 25 °C (Kessler, 1975). The bacterial-bound complexes were washed twice in 2 ml PBA and centrifuged (50 min at 900 g) between washes. Radioactivity in pellets was determined in a Beckman Biogamma 4000 counter. The radioactivity in precipitates formed in the absence of competitor protein was defined as 100% antigen precipitation (0% competition). The radioactivity formed with pre-immune serum was defined as 0% antigen precipitation (100% competition). Competitor antigens used in these experiments were gradient-purified SiSV(SSAV), SiSV(HEL-12) and GALV. Virus was extensively dialysed against PBS, disrupted with 0.1% NP-40 and sonicated to solubilize the virion proteins.

RESULTS

Tests of biological function

Transforming activity of HEL-12 virus was evaluated by infection of normal human foreskin fibroblasts (HFF) and normal marmoset foetal skin fibroblasts (MFS) (Table I). Cultures infected with SiSV yielded characteristic foci of transformed cells, but no foci were observed in parallel HEL-12 virus-infected HFF or MFS cells observed for a minimum of 14 days p.i. and in several experiments after several weeks of serial subcultivation. Infectivity of HEL-12 virus for HFF and MFS was demonstrated by positive reverse transcriptase activity in spent culture fluids. Furthermore, marmoset cells infected with HEL-12 virus induced syncytia formation in a 48 h mixed culture cytopathogenicity (MCC) test with XC cells. In contrast to virus-producing HEL-12 cells (p32), non-producing HEL-12 cells (p6) did not induce syncytia in an MCC test with XC cells (Table I).

Helper activity of HEL-12 virus was demonstrated by rescue of the sarcoma virus genome in SiSV-transformed non-producer marmoset cells (HF/SiSV-NP) following superinfection with supernatants from HEL-12 p32 cells; supernatants from HEL-12 p6 cells did not rescue the sarcoma virus genome. The foci induced by rescued virus, SiSV(HEL-12), were morphologically indistinguishable from SiSV(SSAV)-induced foci.

Helper activity was also investigated by co-cultivation of HF/SSV-NP cells with early passage HEL-12 cells (p6) or HEL-12 cells spontaneously releasing virus (p32). HEL-8 cells, a human embryonic lung cell strain in which C-type virus expression has not been
### Table 1. Evaluation of virus production and HEL-12 helper activity

<table>
<thead>
<tr>
<th>Cell culture</th>
<th>Reverse transcriptase*</th>
<th>Focus formation† (f.f.u./ml)</th>
<th>XC syncytium induction‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>HF</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>HF/SiSV-NP V</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>HF/SiSV-NP VI</td>
<td>-</td>
<td>0</td>
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<tr>
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<td>0</td>
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<tr>
<td>HEL-12 p7</td>
<td>-</td>
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<tr>
<td>HEL-12 p32</td>
<td>+</td>
<td>0</td>
<td>+</td>
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<tr>
<td>HEL-8</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>HF/SiSV-NP V/HEL-12 p32</td>
<td>+</td>
<td>$2 \times 10^4$</td>
<td>+</td>
</tr>
<tr>
<td>HF/SiSV-NP VI/HEL-12 p32</td>
<td>+</td>
<td>$1 \times 10^4$</td>
<td>+</td>
</tr>
<tr>
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<td>-</td>
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<td>-</td>
</tr>
<tr>
<td>HF/SiSV-NP VI/HEL-12 p6</td>
<td>ND</td>
<td>$2.4 \times 10^4$</td>
<td>ND</td>
</tr>
<tr>
<td>HF/SiSV-NP VI/HEL-12 p6</td>
<td>ND</td>
<td>$7 \times 10^4$</td>
<td>ND</td>
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</tbody>
</table>

* Reverse transcriptase assays were performed on concentrated spent culture fluids as previously described (Panem et al. 1975). Activity was measured as the incorporation of $^3$H-dTTP into acid-precipitable counts using the synthetic template poly(rA).oligo(dT)$_{12-18}$. Positive activity was judged relative to a negative control (no virus, generally 300 to 800 ct/min) and a positive control [SiSV(SSAV) adjusted to give 30,000 ct/min incorporation]. Positive activity was judged as threefold higher than the negative control.

† Transformation assays and rescue by co-cultivation were performed as described in Methods.

‡ Induction of XC syncytia by co-cultivation was as described in Methods.

§ ND, Not done.

detected, served as negative control cells. Spent culture fluids were assayed for focus-forming virus after 14 days of co-cultivation. Supernatant fluids from cultures of the cell strains propagated individually did not induce focus formation nor did fluids from cocultures of either clone of HF/SiSV-NP with HEL-8 cells. In contrast to experiments with tissue culture supernatants, rescue of SiSV was demonstrated by both p32 and p6 HEL-12 cells when co-cultivated with either of two clonal strains of HF/SiSV-NP cells (Table 1).

The finding that SiSV was rescued by co-cultivation of HF/SiSV-NP with HEL-12 p6 cells was further explored. HEL-12 p6 cells were found to be negative for production of virus detectable by assay of supernatant reverse transcriptase activity, electron microscopy, XC syncytia induction or the ability of spent culture fluids to rescue SiSV from HF/SiSV-NP cells (Table 1). In addition, control parallel cultures of HEL-12 cells examined for spontaneous virus production at passage 7 were non-productive as judged by assay of reverse transcriptase activity. The expression of virus envelope antigens by p6 cells was also evaluated. In complement-dependent serum cytotoxicity tests, no SSAV-related envelope antigens were detected at the cell surface using goat anti-SiSV(SSAV) serum under conditions where HF/SiSV(SSAV) and HF/SiSV-NP(HEL-12) were lysed (data not shown). Previous studies have shown that HEL-12 p6 cells were producing either no virus antigen or amounts which were not detectable by cytoplasmic immunofluorescence (Panem et al. 1977). However, in these earlier studies HEL-12 cells began to spontaneously express SiSV(SSAV)-related antigen between passages 8 and 9 (Panem et al. 1977). It was also found that virus may be induced from HEL-12 cells by iododeoxyuridine or 2-deoxyglucose treatment when antigen expression, as measured by indirect immunofluorescence, begins (Prochownik et al. 1979).

**Comparative neutralization and serum cytotoxicity tests**

The antigenic properties of HEL-12 virus envelope proteins were compared to those of GALV and SSAV by measuring inhibition of focus induction by SiSV(HEL-12), SiSV-(GALV) and SiSV(SSAV) in serum neutralization tests. These stocks contained comparable helper:transforming virus ratios as determined by end-point dilution. Samples of each virus
Fig. 1. Neutralization of HEL-12, SSAV and GALV pseudotypes of SiSV with (a) a neutralizing goat anti-SiSV(SSAV) serum or (b) a rabbit antiserum prepared against purified gp70 of SSAV. Surviving virus (Vs) was measured by focus assay on HFF cells. The surviving fraction, Vs/Vo, was calculated relative to virus (Vo) incubated in the absence of antiserum. ○—○, SiSV(HEL-12); •—•, SiSV(SSAV); □—□, SiSV(GALV).

(100 f.f.u.) were reacted with serial dilutions of antiserum. The antisera used were a hyperimmune goat anti-SiSV(SSAV) serum (Fig. 1a) and a rabbit antiserum specific for SSAV gp70 (Fig. 1b). Both sera exhibit antibodies primarily directed towards type-specific antigenic determinants of SSAV envelope proteins. Results of neutralization tests were analysed by plotting surviving fraction (Vs/Vo) versus antiserum dilution. As shown in Fig. 1 (a, b), SiSV(SSAV) and SiSV(HEL-12) were clearly distinguished from SiSV(GALV) with both antisera. In contrast, SiSV(SSAV) and SiSV(HEL-12) had identical 50% endpoint titres and patterns of neutralization. Using goat anti-SiSV serum, a neutralization titre of 1:80 was demonstrated with SiSV(GALV) and a titre of 1:2048 with SiSV(HEL-12) or SiSV(SSAV). Even greater type-specificity was exhibited by the rabbit anti-SSAV gp70 (Fig. 1b). Identical results were obtained using SSV(HEL-12) pseudotypes from p6 or p32 HEL-12 cells.

The time course of neutralization of focus formation by SiSV(SSAV), SiSV(HEL-12) and SiSV(GALV) was examined over a 4 h period with anti-SiSV(SSAV) diluted 1:1000. SiSV(GALV) was not neutralized, whereas SiSV(SSAV) and SiSV(HEL-12) exhibited similar kinetics and extent of neutralization (data not shown). Thus antigenic determinants on HEL-12 gp70 and SSAV gp70 were not distinguished in neutralization experiments with these antisera.

In a complement-dependent serum cytotoxicity test, neutralizing goat anti-SiSV(SSAV) serum killed HF/SiSV(SSAV) and HF/SiSV(HEL-12) cells but not HF/SiSV-NP cells or HF/GALV cells (Fig. 2). These results again indicated that the virus envelope proteins of SSAV and HEL-12 virus are antigenically related.

Comparison of virus antigens by radioimmunoassays (RIA)

Unique and common envelope gp70 antigenic determinants of SiSV(SSAV) and SiSV-(HEL-12) could be demonstrated in sensitive competition radioimmunassays. Fig. 3 presents homologous and heterologous RIAs for SSAV gp70 using disrupted, iodinated SiSV-(SSAV) and anti-SiSV(SSAV) or anti-HEL-12 sera, respectively. In the homologous assay (Fig. 3a), SiSV(SSAV) completely displaced the labelled SiSV(SSAV) antigen whereas
HEL-12 virus gp70

Fig. 2. Comparison of SSAV and HEL-12 virus envelope proteins by complement-dependent serum cytotoxicity. $^{51}$Cr-labelled cells, HF/SiSV(SSAV) (●—●), HF/SiSV-NP(HEL-12) (○—○), HF/SiSV-NP (△—△) and HF/GALV (□—□) were incubated with fourfold dilutions of goat anti-SiSV(SSAV) serum in the presence of complement. $^{51}$Cr release was measured and percent cytotoxicity calculated as described in Methods.

Fig. 3. Homologous and heterologous competition RIA for SSAV gp70. The ability of unlabelled viruses to compete with $^{125}$I-SiSV(SSAV) antigen for a limiting dilution of antiserum was measured as described in Methods. Competitors were HEL-12 virus (○—○) and SiSV(SSAV) (●—●). The data are presented as the average results of duplicate determinations. Antiserum used were (a) goat anti-SiSV(SSAV) and (b) rabbit anti-HEL-12 virus.

HEL-12 competed for only 20%. In a heterologous assay using antiserum specific for HEL-12 gp70, SiSV(HEL-12) competed with a slope and extent of competition similar to SiSV(SSAV) (Fig. 3b), indicating that the limited ability of HEL-12 to compete in the type-specific assay for SiSV(SSAV) gp70 cannot be attributed to loss of HEL-12 gp70 during purification. Although the results of these assays imply differences in the gp70s, interpretation is limited by the use of multivalent reagents. Therefore, these results were confirmed by monospecific RIA.

Fig. 4 presents competition radioimmunoassays directed against $^{125}$I-labelled SSAV gp70. In the homologous RIA, SiSV(SSAV) competed completely with 0.048 μg of disrupted virus required for 50% competition. In contrast, GALV and SiSV(HEL-12) required 10 and 100 μg, respectively, for 50% competition. GALV competition reached a plateau at 60%. SiSV(HEL-12) did not reach a plateau in this assay and the slope of its competition curve was different, as determined by regression analysis, from that of SiSV(SSAV). The RIA data suggest differences in the type-specific antigenic determinants of HEL-12, SSAV and GALV gp70s.

Interspecies gp70 determinants were investigated with a competition radioimmunoassay employing SiSV(SSAV) gp70 and antiserum specific for FLV gp70 (Fig. 5). SiSV(SSAV) competed for 100% of the labelled antigen; 0.3 μg protein was needed for 50% competition. In contrast, SiSV(HEL-12) reached plateau levels at 20% competition with 10 μg protein, and 10 μg of GALV competed to 50%. These results also suggested that antigenic determinants on HEL-12 gp70 and SSAV gp70 are similar but not identical.
Fig. 4. Homologous competition for purified SSAV gp70. The antigenic relations of the gp70s of Si- 
SV(SSAV), SiSV(HEL-12) and GALV were examined in competition radioimmunoassays using 
\(^{125}\text{I}-\text{SSAV gp70 (4000 ct/min) as the antigen and goat anti-SiSV(SSAV) serum. For each com-
petition 50 \mu l of antiserum was added at a dilution (1:200) to precipitate 50\% of the input antigen 
radioactivity. Competitor proteins were NP-40-disrupted gradient-purified viruses: O—O, SiSV-
(SSAV); O—O, SiSV(HEL-12); □—□, GALV. The average ct/min \(^{125}\text{I}-\text{antigen precipitated 
by duplicate samples and the percent of competition are presented: 0\% competition is defined as the 
ct/min obtained with pre-immune serum, 100\% competition as the ct/min obtained with immune 
serum in the absence of competitor.}

Fig. 5. Heterologous RIA for purified SSAV gp70. The relatedness of interspecies gp70 antigenic 
determinants was examined in competition radioimmunoassays with \(^{125}\text{I}-\text{SSAV gp70 (4000 ct/min) 
as described for Fig. 4, but using rabbit anti-FLV gp70 at a 1:100 dilution. Competitor proteins were 
NP-40-disrupted gradient-purified viruses: O—O, SiSV(SSAV); O—O, SiSV(HEL-12); □—□, GALV.}

DISCUSSION

These experiments compare biological properties of SiSV(SSAV) and HEL-12 not 
previously investigated and represent the first quantitative comparison of the antigenic 
determinants of the envelope glycoproteins of HEL-12 virus with those of SiSV(SSAV) and 
GALV. On the basis of indirect cytoplasmic immunofluorescence, immunodiffusion, 
inhibition of reverse transcriptase assays and molecular hybridization experiments, HEL-12 
virus was previously shown to belong to the group of exogenous primate retroviruses of 
which SSAV and GALV are prototypes (Panem et al. 1975, 1976, 1977). Studies reported 
here demonstrate that HEL-12 virus is non-transforming, will induce syncytia with XC 
cells and can act as a helper for replication-defective SiSV.

Previous studies demonstrated that early passage HEL-12 cells contain SSAV-related
proviral DNA (Prochownik et al. 1979) but no detectable virus antigen expression or virus production in the first 25 days after reinitiation of cultivation of primary cells stored in liquid nitrogen. Spontaneous release of virus was coordinated with duration of in vitro cultivation. Before spontaneous virus production HEL-12 virus can be elicited from antigen-positive cells by treatment with iododeoxyuridine or 2-deoxyglucose (Prochownik et al. 1979). We have now demonstrated that co-cultivation of non-virus-producing early passage (p6) HEL-12 cells with HF/SiSV-NP can reproducibly result in rescue of the SiSV genome. Although we cannot exclude the fact that HEL-12 virus was released from early passage cells at exceptionally low levels, p6 cells were negative for virus production by several criteria. Furthermore, HEL-12 p7 cells propagated in parallel remained virus-negative. Although no virus-specific envelope antigens were detectable on the surface of p6 cells, previous immunofluorescence tests indicated that low levels of cytoplasmic antigen may be present. It is possible that co-cultivation alters a host-cell restriction and/or allows activation of the latent HEL-12 virus genome. This phenomenon, the molecular mechanism of which has yet to be elucidated in any system, is not unique and has in fact facilitated the isolation of several primate retroviruses (Benveniste et al. 1974; Todaro et al. 1978; Rabin et al. 1979).

When the antigenic properties of HEL-12 gp70 were quantitatively compared to those of SSAV and GALV by serum neutralization assays, SiSV(SSAV) and SiSV(HEL-12) yielded identical patterns of neutralization distinct from SiSV(GALV). Analysis of the kinetics of neutralization also failed to distinguish SiSV(HEL-12) from SiSV(SSAV), but again clearly differentiated SiSV(HEL-12) from SiSV(GALV). As has been shown for closely related murine retroviruses which could not be distinguished by serum neutralization tests (Hino et al. 1976), antigenic cross-reactivity, but non-identity of HEL-12 and SSAV gp70s was demonstrated in competition radioimmunoassays.

Neither HEL-12 virus nor SiSV(HEL-12) grown in marmoset cells was able to compete significantly in a type-specific homologous radioimmunoassay for SiSV(SSAV) gp70. However, in heterologous radioimmunoassays, SiSV(HEL-12) and SiSV(SSAV) yielded similar competition profiles, indicating that HEL-12 and SSAV have common as well as distinct gp70 antigenic determinants. In addition to differences in type-specific gp70 determinants, a radioimmunoassay using labelled SiSV(SSAV) gp70 and anti-FLV serum revealed differences between SiSV(HEL-12) gp70 and SiSV(SSAV) gp70 interspecies antigenic determinants.

These antigenic differences between HEL-12 gp70 and SSAV gp70 cannot be attributed to differences in patterns of host cell glycosylation because (i) identical results were obtained with HEL-12 or SiSV(SSAV) propagated in human or marmoset cells and (ii) antisera raised to SiSV grown in goat or marmoset cells gave identical results. Furthermore, it has been reported that differential glycosylation did not affect the antigenic specificity of other retrovirus gp70s (Bolognesi et al. 1975). It is of interest that the env gene and gp70 of SSAV appear to be very stable; antigenic variability of SSAV gp70 following passage over the past 5 years in cells derived from a number of species has never been demonstrated by neutralization or RIA.

In earlier studies it was reported that HEL-12 cells express virus antigens related to baboon endogenous virus (BaEV), as well as to the antigenically distinct SiSV/GALV groups (Panem et al. 1976, 1977). Nevertheless, it is unlikely that the differences between the gp70s of HEL-12 and SSAV observed in RIAs are related to the existence of a mixed HEL-12 virus population comprised of both SSAV- and BaEV-related viruses, as studies indicate that BaEV replication is restricted in marmoset cells used to propagate SiSV-(HEL-12) for these studies (Bergholz et al. 1977a). Homogeneity of the HEL-12 virus pool is also suggested by the absence of breakthrough foci in serum neutralization tests in which
HFF cells, permissive for BaEV, were used for assay of surviving virus. Ribonuclease T1 oligonucleotide mapping of HEL-12 70S RNA failed to demonstrate HEL-12 virus RNA oligonucleotides that were BaEV-specific, while comparison of HEL-12 and SiSV(SSAV) RNA fingerprints demonstrated that the respective virus RNAs were distinguished only by a total of four large oligonucleotides (E. Hefti & S. Panem, unpublished data). Preliminary nucleic acid hybridization experiments suggest that HEL-12 and SSAV are at least 90% homologous (R. G. Smith, personal communication). These findings are in agreement with the results of studies of antigenic relatedness reported here, which demonstrate only minor differences in the envelope protein of SSAV and HEL-12 virus. Experiments of Reitz et al. (1976) have demonstrated that viruses of the SSAV/GALV group show the greatest sequence diversity at the 3' end of the virus genome, i.e. the region thought to code for gp70.

In summary, experiments reported here demonstrate that HEL-12 virus has biological properties similar to SSAV and GALV and is indistinguishable from SSAV in serum neutralization or cytotoxicity assays which clearly distinguish SSAV from GALV. At the same time RIA experiments provide the first evidence that the gp70 of HEL-12 is closely related but not identical to gp70 of SSAV or GALV. Minor differences in virus envelope proteins may have important biological implications.

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REFERENCES


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