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Cytocidal Effect Caused by the Envelope Glycoprotein of a Newly Isolated Avian Hemangioma-Inducing Retrovirus

NITZAN RESNICK-ROGUEL,[1] HAIM BURSTEIN,[1] JUDITH HAMBURGER,[1] AMOS PANET,[2] AMIRAM ELDOR,[3] ISRAEL VLODAVSKY,[4] AND MOSHE KOTLER[1]*

Departments of Molecular Genetics,[1] Virology,[2] Hematology,[3] and Oncology,[4] Hadassah Medical School, The Hebrew University, P.O. Box 1172, Jerusalem 91010, Israel

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We isolated a field strain of avian hemangioma retrovirus (AHV) which induces a cytopathic effect (CPE) on cultured avian and mammalian cells shortly after infection. The kinetics of cell killing were dependent on the multiplicity of infection. The CPE on avian and mammalian cells was independent of virus replication, because UV-irradiated virus led to cell death as well. Biochemical and genetic experiments indicated that AHV *env* gene products were responsible for the CPE. Partially purified AHV envelope glycoproteins (gp85), but not those of the Rous sarcoma virus Prague C strain, induced a CPE. Rous-associated virus type 1, in which the *env* region was replaced by the AHV gp85 region, induced a CPE on avian and mammalian cultured cells. Therefore, we suggest that CPE is induced by AHV via interaction between viral gp85 and the cell membrane. This mode of CPE is unique among avian sarcoma-leukemia viruses.

Induction of a cytopathic effect (CPE) by viruses occurs by various mechanisms. Interaction between some viruses and the cell membrane is sufficient for induction of a CPE (12, 13, 15), whereas other viruses cause cell death only after penetrating the cells. Accumulation of viral nucleic acid, proteins or particles, interrupts the host cell metabolism (4, 16, 17, 26). Finally, a CPE may also be induced during viral budding or expression of viral proteins on the outer cell membrane (6, 14).

Avian sarcoma-leukemia viruses (ASLV) are classified into subgroups A through G according to host range, antigenicity, and ability to interfere with other ASLV; all of these properties are determined by the viral envelope gene (*env*) (7). *env* gene products are initially synthesized as a precursor protein with a molecular mass of 95 kilodaltons which is cleaved into two glycoproteins (gp85 and gp37). These two glycoproteins are linked by disulfide bonds; the glycoprotein complex is anchored to the virion membrane by gp37, whereas gp85 protrudes outside the virion membrane (6).

Detailed analysis of gp85 has revealed a conserved structure with several variable domains (Vr1, Vr2, hr1, hr2, and Vr3) which determine the host range of the virus and the abilities of certain subgroups to infect mammalian cells, albeit inefficiently (7, 8).

Viruses belonging to subgroups B, D, and F are capable of inducing a CPE in permissive cells, beginning 2 to 3 days postinfection (p.i.), for 8 days. During this period, cells die because of accumulation of viral DNA, probably as a result of superinfection. This phenomenon suggests that envelope glycoproteins of viral subgroups B, D, and F are unable to prevent superinfection and therefore play an indirect role in CPE induction. Thus, these viruses cause a CPE after penetrating host cells and may therefore be classified as members of the second group of CPE-inducing viruses (26–28).

Avian hemangioma virus (AHV) is a field virus recently isolated by us from spontaneous lesions in layer hens (3). The name of the virus indicates the tumor from which this virus was isolated and its potential to induce similar lesions in 30% of chickens inoculated on the day of hatching (3; H. Burstein et al., submitted for publication). In this report, we demonstrate that AHV induces a CPE in a wide variety of both avian and mammalian cells. On the basis of the following results, we propose that the *env* product plays a direct role in the induction of a CPE. (i) A CPE can be observed within hours p.i. (ii) UV-inactivated virus induces a CPE. (iii) A CPE is induced by partially purified viral envelope glycoproteins. (iv) Recombinant Rous-associated virus type 1 (RAV-1), containing most of the coding sequence for gp85 of AHV, is converted into a cytopathic virus. We conclude that CPE is induced by AHV in cultured cells without penetration of the cells and that the *env* glycoproteins are sufficient for cell killing.

MATERIALS AND METHODS

Cells. Chicken embryo fibroblasts (CEF) were prepared from eggs obtained from SPAFAS, Inc., Norwich, Conn. The QT-6, BHK, and BSC-1 cell lines, derived from quail, monkeys, and hamsters, respectively, and CEF were grown in Dulbecco modified Eagle medium with 10% tryptose phosphate and 10% heat-inactivated fetal bovine serum as described previously (11). Bovine aortic endothelial cells (BAEC) prepared from an adult aortic arch (10) were grown in Dulbecco modified Eagle medium supplemented with 10% inactivated newborn bovine serum.

Viruses. AHV was isolated by cocultivation of spontaneous chicken tumors with CEF and was further cloned by endpoint dilution on QT-6 cells as described before (3). The titer of the virus (infectious particles) was determined by endpoint dilution. The same stock virus was assayed for reverse transcriptase (RT) activity (9) and used for further experiments as a standard stock. QT-6 cells were usually used to prepare stock virus, because a CPE was induced only in the presence of a high virus titer. Hirt extracts prepared from infected QT-6 cells were used as a source of unintegrated provirus for cloning of AHV DNA into gtWESB, which was further subcloned into the pSP-65 vector (Burstein et al., manuscript in preparation). CEF and QT-6 cells were transfected with a DNA clone of AHV

* Corresponding author.

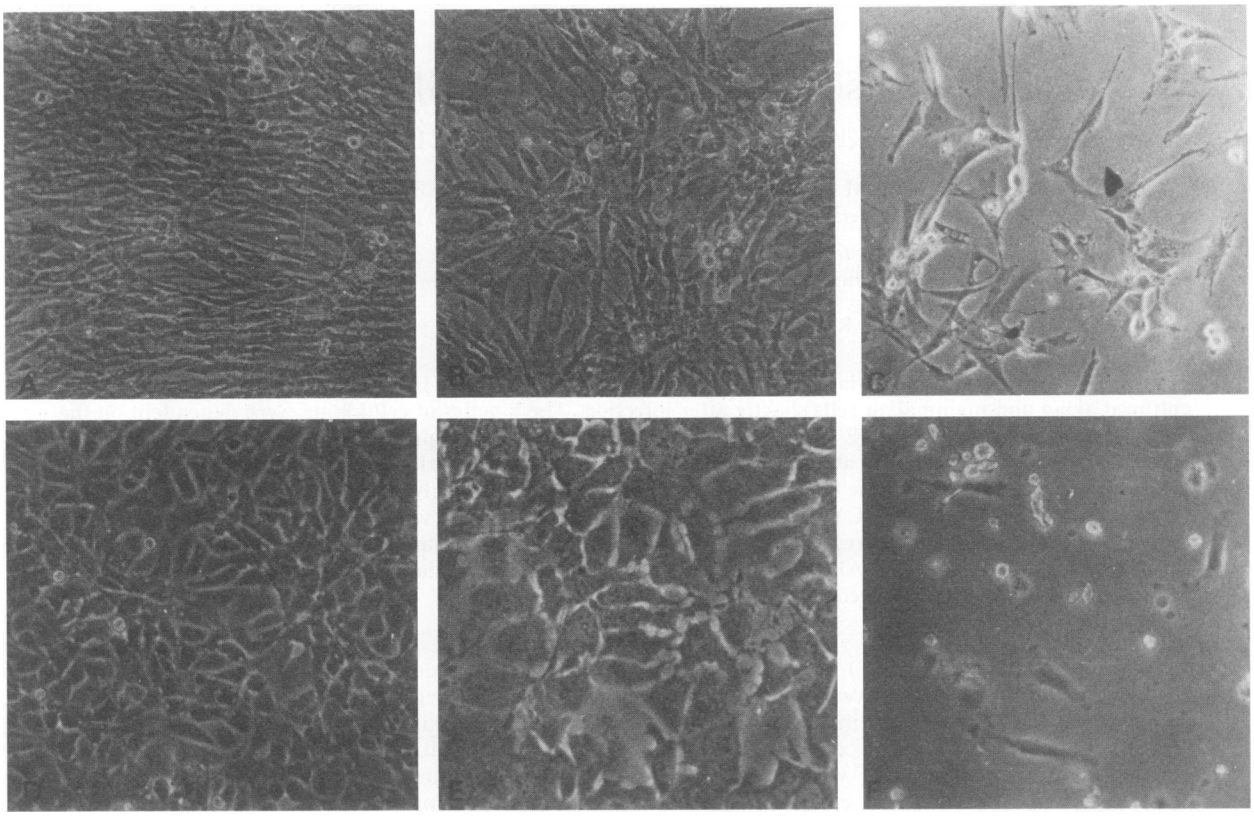

FIG. 1. CPE induced by AHV on CEF and BAEC. Cells were infected with the virus at MOIs of 1 and 25. Pictures were taken at 24 h p.i. Panels: A to C, CEF cultures; D to F, BAEC cultures; A and D, noninfected cells; B and E, cells infected at an MOI of 1; C and F, cells infected at an MOI of 25.

(AHV_{52}). Only a small proportion of the cells was stably transfected by this procedure. Therefore, the transfected cells were maintained in culture for 10 to 15 days. During this period, AHV_{52} achieved a high titer and a CPE was observed. The avian sarcoma virus Prague C strain (Pr-C), was propagated in CEF; focus-purified virus was used in all experiments.

Concentration of viruses. Culture media harvested from infected cell cultures were centrifuged for 10 min at 10,000 × *g* to remove cell debris. Virus was sedimented by centrifugation for 1 h at 100,000 × *g*, and pellets were suspended in phosphate-buffered saline (PBS). The suspended virus was further purified in 15 to 65% sucrose gradients, and fractions close to 1.16 g/ml were diluted in PBS and sedimented for 1 h at 100,000 × *g*.

Irradiation of the virus. AHV was transferred into a watch glass plate and UV irradiated for 40 min by a Mineralicht lamp (model UVGL-58) at 254 nm from a distance of 35 cm.

Isolation of viral glycoproteins. The isolation procedure is a modification of the method described by Scheid et al. (22). The pelleted virus was suspended in an equal volume of PBS containing 20% Triton X-100 by gentle mixing for 20 min at room temperature. The suspensions were then centrifuged in a Beckman Airfuge (22 lb/in^2 for 30 min). The supernatants were collected, vigorously mixed with 6 volumes of cold butanol, and incubated on ice for 30 min. Suspensions were centrifuged for 10 min at 9,000 × *g*, and pellets containing the viral glycoproteins were washed three times in ether and suspended in PBS.

Portions of viral glycoprotein preparations were fractionated on a 12% polyacrylamide-sodium dodecyl sulfate gel. Proteins were stained by Coomassie blue, and the glycoproteins were further detected specifically by concanavalin A-horseradish peroxidase staining as described by Wood and Sarinana (30).

Construction of recombinant viruses. Plasmid (pRAVIb) containing the permuted RAV-1 genome (19) was cleaved with restriction enzymes *Sal*I and *Kpn*I as instructed by the supplier. A plasmid containing the AHV genome ($pAHV_{52}$) was also digested with enzymes *Kpn*I and *Sal*I. The 1.0-kilobase *Kpn*I-*Sal*I fragment (most of the gp85 region of AHV) was introduced into pRAV-1, and the reciprocal exchange was performed as well (see Fig. 5C).

DNA transfection. The permutated viral DNA inserts were cleaved from the vector by *Sal*I endonuclease and ligated to construct complete viral genomes. QT-6 cells were transfected with the viral DNA by the calcium phosphate method (5). The presence of viruses in the culture media was detected by RT assay (9).

RESULTS

AHV induces a CPE in a wide range of cultured cells. AHV induced a CPE in chicken, turkey, goose, and QT-6 cells. The cytocidal effect induced by AHV was not limited to avian cells and was also observed in mammalian cells, namely, cultured BSC-1 and BHK cells and BAEC (Fig. 1). No CPE was observed in cultures of avian and mammalian cells treated with RAV-1, RAV-2, or Pr-C (multiplicity of infection [MOI], about 5). AHV-infected CEF and BAEC became elongated and vacuoles appeared in the cytoplasm before cell death (Fig. 1).

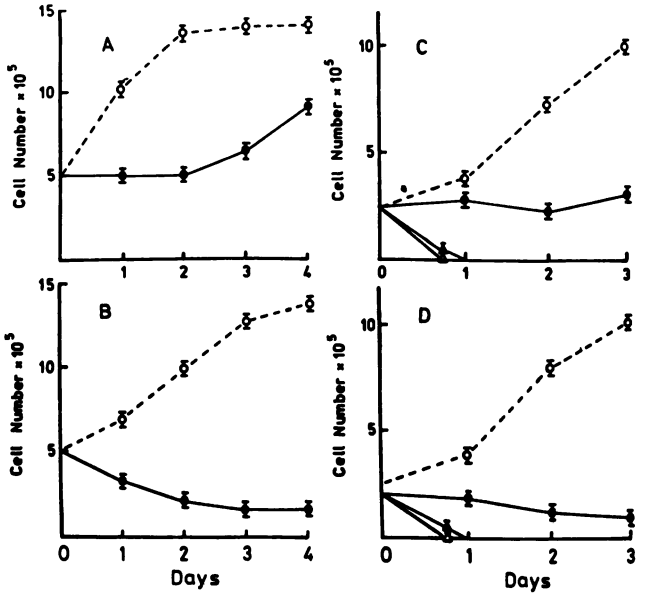

FIG. 2. Kinetics of CPE induced by AHV and AHV_{52} in CEF and BAEC. BAEC (A and C) and CEF (B and D) were infected with AHV (A and B) or AHV_{52} (C and D). Cells were harvested at the indicated intervals, and the numbers of viable cells were determined by trypan blue staining. For panels C and D, AHV_{52} was applied to the cells at MOIs of 2 (●), 10 (▲), and 40 (△). ○, Noninfected control cells.

Kinetics of the cytocidal effect caused by AHV. Figure 2 shows the numbers of surviving BAEC and CEF after AHV infection, as determined by trypan blue staining. The number of viable CEF decreased constantly, whereas the number of endothelial cells decreased or remained constant throughout the first 2 days p.i., after which the cells began to proliferate. The constant number of BAEC probably reflects a delicate equilibrium between death and proliferation of cells in culture. The difference between BAEC and CEF appears to reflect the ability of AHV to replicate and spread in avian cell cultures but not in cultured mammalian cells. This conclusion was further substantiated by the absence of RT activity in concentrated media harvested from the endothelial cells (data not shown).

A similar killing effect (Fig. 2A and B) was demonstrated by using AHV_{52} derived from cloned proviral DNA. The results indicate that the cloned AHV (AHV_{52}) is identical to the field virus isolated from spontaneous hemangiomas in terms of this specific biological property. In addition, genomic RNA oligonucleotide fingerprint analysis and proviral DNA restriction enzyme mapping confirmed that the two viruses were identical (Burstein et al., manuscript in preparation).

The cytocidal effect caused by infection of CEF and BAEC with increased virus concentrations is presented in Fig. 2C and D. AHV at MOIs of 20 and 80 caused a dramatic and rapid CPE in both types of cells. Increased numbers of nonviable cells were observed as early as 12 to 18 h p.i. In contrast, infection of the same cultures with similar concentrations of Pr-C virus caused no CPE. This rapid cytocidal effect suggests that the virus affects cells during the initial steps of infection, before viral multiplication.

Induction of a CPE by inactivated AHV. AHV was inactivated by UV irradiation and added to CEF and BAEC cultures. The data presented in Fig. 3 indicate that inactivated AHV caused a CPE in both CEF and BAEC cultures. Cell killing was observed as early as 24 h posttreatment. To verify complete inactivation of the virus, treated CEF cultures were grown and passaged twice in 16 days. The media were harvested and tested for the presence of viral particles by RT assay (9). Although as few as 10^3 focus-forming units/ml of virus can be detected by this method, no enzymatic activity was detected. Unlike CEF infected with active AHV (Fig. 2), chicken cells treated with UV-irradiated AHV recovered and resumed proliferation by 48 h p.i.; this is an additional indication that the irradiated virus is unable to replicate. These results support our hypothesis that a CPE can be induced by AHV without virus replication.

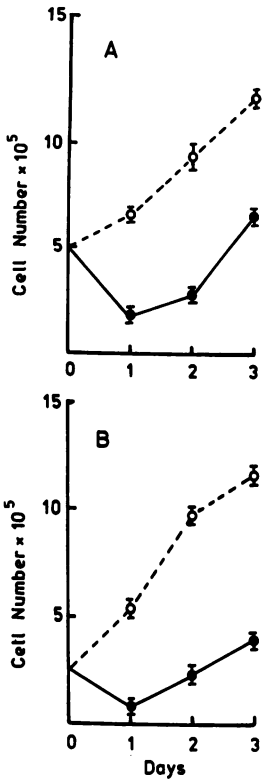

FIG. 3. Induction of a CPE by UV-inactivated AHV_{52}. BAEC (A) or CEF (B) were mock infected (○) or infected with AHV_{52} (MOI, 2) (●), and the numbers of viable cells were determined by trypan blue staining.

A CPE is specifically induced by AHV glycoproteins. The foregoing results encouraged us to investigate the possibility that isolated AHV glycoproteins were able to induce a CPE in cultured cells. The viral glycoproteins were partially purified by the method described by Scheid et al. (22), as revealed by polyacrylamide gel electrophoresis (Fig. 4). The gel was further stained with concanavalin A-horseradish peroxidase, a specific staining for glycoproteins, verifying that the 85-kilodalton protein was indeed a glycoprotein (data not shown). Approximately 2.5% of the whole Rous sarcoma virus virion consists of gp85 (1). Accordingly, equivalent amounts of isolated glycoproteins or virus inoculum were added to cultured cells (300 ng of glycoproteins, equivalent to about 10^5 focus-forming units of Pr-C virus). The Pr-C virus and its isolated glycoproteins were used as a control. The results, summarized in Table 1, indicate that the percentage of surviving cells at 24 h after treatment with glycoproteins was reduced to 58% in CEF and 39% in BAEC

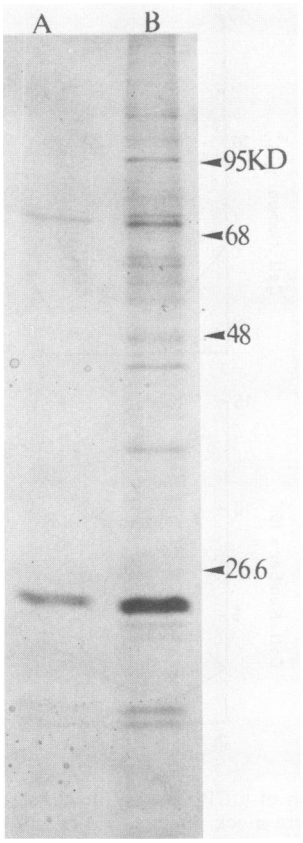

FIG. 4. Polyacrylamide gel electrophoresis of AHV glycoproteins stained with Coomassie blue. Lanes: A, partially purified preparation of AHV glycoproteins used for treating cultured cells (Table 1); B, whole virus. KD, Kilodaltons.

cultures. Similar effects were observed after treatment of these cells for 24 h with an equivalent inoculum of infectious virus (44 and 35%, respectively). However, CEF and BAEC treated with either Pr-C virus or its isolated glycoproteins remained unaffected (Table 1). It appears that partially purified AHV glycoproteins induce a CPE in both avian and mammalian cells.

Induction of a CPE by recombinant RAV-1 containing the AHV *env* gene. RAV-1, a member of ASLV subgroup A, is a noncytopathic virus. To verify that the cytopathogenicity of AHV is induced by the *env* gene products, we replaced most of the RAV-1 gp85 DNA coding sequences with those of AHV and vice versa (Fig. 5). The *Kpn*I-*Sal*I restricted DNA fragments containing the major portion of the gp85-coding sequences were cleaved from both RAV-1 and AHV_{52} and reciprocally ligated into the deleted proviruses. This exchange of genetic information could be accomplished because RAV-1 and AHV_{52} have very similar restriction enzyme maps (Burstein et al., manuscript in preparation). The recombinant proviral DNAs were transfected into QT-6

TABLE 1. Induction of a CPE by purified AHV_{52} envelope glycoproteins[a]

Treatment of cells	Protein content (ng)	% of viable cells	
		CEF	BAEC
None		100	100
AHV_{52} infection	310	58	39
AHV_{52} envelope glycoproteins	1.6	44	38
	0.8	47	35
	0.16	63	63
Pr-C virus infection	400	100	93
Pr-C envelope glycoproteins	10	100	95

[a] CEF and BAEC cultures were washed twice with PBS and treated with either viral preparations or glycoproteins. Medium was added to the cells, and at 24 h posttreatment the number of viable cells was determined by trypan blue staining. The gp85 content is 2.5% of whole virion; therefore, the 310 ng-protein content of the whole virus is equivalent to 8 ng of purified gp85.

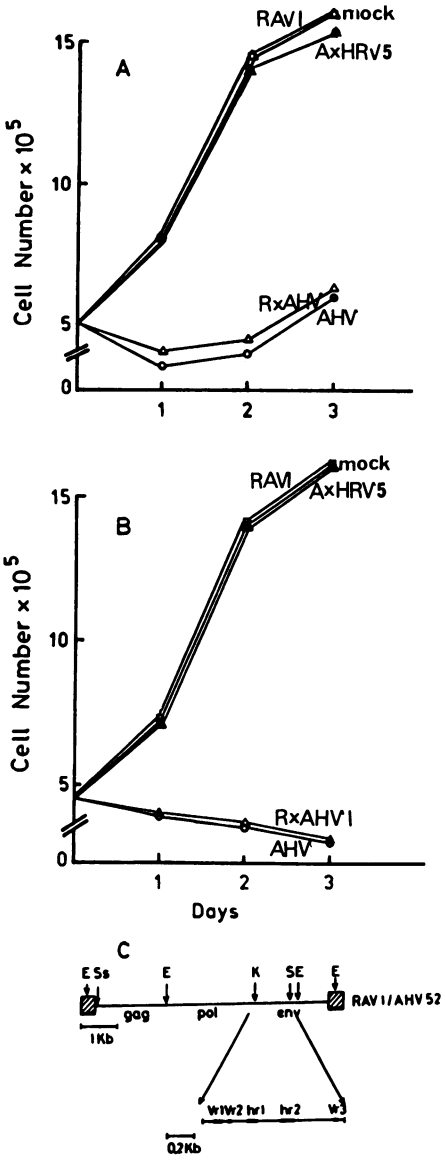

FIG. 5. Induction (A and B) of a CPE by recombinant virus RxAHV-1, which contains AHV_{52} gp85. BAEC (A) and CEF (B) cultures were infected with RAV-1 (□), AHV_{52} (○), RxAHV-1 (△), or AxHRV-5 (▲) at an MOI of 2 or mock infected (◇). At 1 to 4 days p.i., the numbers of viable cells were determined. (C) Construction of recombinants RxAHV-1 and AxHRV-5. The restriction map of RAV-1 and AHV is on the upper line. A *Kpn*I-*Sal*I fragment of RAV-1, which contains most of gp85 (bottom line), was replaced by the homologous fragment of AHV_{52} to construct RxAHV-1. A reciprocal exchange was made to construct recombinant AxHRV-5 (AHV_{52} which carries RAV-1 gp85). Kb, Kilobase; E, *Eco*RI; Ss, *Sst*I; K, *Kpn*I; S, *Sal*I.

cells, and the presence of replicating virus was verified by RT activity in the culture media.

Both recombinant viruses were used to infect avian (CEF) and mammalian (BAEC) cells. The results (Fig. 5) demonstrate that recombinant RAV-1 expressing AHV_{52} gp85 (termed RxAHV-1) caused a CPE in both avian and mammalian cells similar to the effect induced by the parental strain AHV_{52}. The reciprocal recombinant, AHV_{52} provirus with RAV-1 gp85-coding sequences (termed AxHRV-5), caused no CPE in these cultures. The CPE caused by recombinant RxAHV-1 in both avian and mammalian cells was apparent shortly after infection, and the results obtained with the purified glycoproteins support our assumption that the *env* gene product of AHV is responsible for the cytopathogenicity of this virus.

DISCUSSION

AHV causes a CPE in a variety of avian and mammalian cultured cells, including fibroblasts and epithelial and endothelial cells. The kinetics of cell killing correlate with the viral MOI. The results presented in this report indicate that the AHV *env* gene product, possibly gp85, is responsible for the cytopathogenicity. Moreover, neither viral replication nor viral gene expression is essential for induction of a CPE.

The CPE caused by AHV is distinguished from that caused by the other ASLV of subgroups B, D, and F by the following characteristics: (i) a CPE induced by AHV appears very rapidly and is correlated with the MOI; (ii) subgroup B viruses induce a CPE specifically in avian cells, whereas AHV is cytocidal for mammalian cells as well; (iii) unintegrated DNA molecules accumulate in CEF shortly after infection with ASLV, probably as a result of superinfection (26). In contrast, replication of AHV is not a prerequisite for the CPE induced by the virus.

AHV is probably a RAV-1 variant, as suggested by its host range (R. F. Smith, personal communication) and the *env* gene sequence (Burstein et al., manuscript in preparation). This finding is surprising in view of the ability of AHV to induce a CPE. Moreover, a CPE was induced by AHV glycoprotein molecules. A specific domain of AHV gp85 responsible for the cytopathogenicity has not been defined; construction of recombinant clones between RAV-1 and AHV will enable us to localize the region responsible for the cytopathogenicity.

By what mechanism does gp85 determine viral cytopathogenicity? It was suggested that gp85 of ASLV subgroups B, D, and F play an indirect role in cytopathogenicity by allowing superinfection, as the result of which proviral DNA molecules accumulate in infected cells (26). With AHV, which induces a CPE by both UV-inactivated virus and isolated gp85 molecules, we assume that some interaction between gp85 and a cell surface component (receptor) leads to cell death. Our finding that AHV induces a CPE in both avian and mammalian cells suggests that the cell surface component (receptor) which is recognized by and interacts with AHV gp85 might be conserved throughout evolution.

The *env* gene products play an important role in retroviral pathogenicity. Recently, it was shown that recombinant avian leukosis virus clones in which the *env* genes of RAV-1 and RAV-0 were exchanged gained or lost pathogenicity depending on the inserted *env* gene (2). Ring-necked pheasant virus, which also induces a CPE in vitro, lost the ability to cause angiosarcomas when its *env* gene was replaced by the RAV-1 *env* gene (23).

Involvement of the *env* gene in pathogenicity is not restricted to ASLV. Recent reports conclude that the envelope glycoproteins of several mammalian retroviruses, such as spleen focus-forming virus and human immunodeficiency virus type I, are involved in the induction of malignant diseases (20, 24, 25, 29). In human immunodeficiency virus type I-infected patients, depletion of the T4 helper cell population is thought to result from cell death related to a CPE caused by this virus in vivo (24, 25). The hemangioma induced in chickens by AHV has some histological resemblance to Kaposi's sarcoma, which is associated with acquired immunodeficiency syndrome (18, 21). In addition, AHV-treated endothelial cells share some biological properties with cells isolated from Kaposi's sarcoma tumors, including enhanced expression of lymphokines and growth factors (N. Resnick-Roguel et al., manuscript in preparation).

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LITERATURE CITED

1. **Bolognesi, D. P., H. Bauer, H. Gelderblom, and G. Huper.** 1972. Polypeptides of avian tumor viruses—components of the viral envelope. Virology **47**:551–566.
2. **Brown, D. W., B. P. Blais, and H. L. Robinson.** 1988. Long terminal repeat (LTR) sequences, *env*, and a region near the 5′ LTR influence the pathogenic potential of recombinants between Rous-associated virus types 0 and 1. J. Virol. **62**:3431–3437.
3. **Burstein, H., M. Gilead, U. Bendheim, and M. Kotler.** 1984. Viral aetiology of hemangiosarcoma outbreaks among layer hens. Avian Pathol. **13**:715–726.
4. **Choppin, P. W., and A. Scheid.** 1980. The role of viral envelope glycoprotein in adsorption, penetration and pathogenicity of viruses. Rev. Infect. Dis. **2**:4–61.
5. **Demetrios, A. S., and N. M. Welkie.** 1984. Expression of exogenous DNA in mammalian cells, p. 1–45. *In* B. D. Hames and S. J. Higgins (ed.), Transcription and translation. IRL Press, Inc., Boca Raton, Fla.
6. **Dickson, C., R. Eisenman, H. Fan, E. Hunter, and N. Teich.** 1982. Protein biosynthesis and assembly, p. 513–648. *In* R. Weiss (ed.) RNA tumor viruses, 1st ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
7. **Dorner, A. J., and J. M. Coffin.** 1986. Determinants for receptor interaction and cell killing on avian retrovirus glycoprotein gp 85. Cell **45**:365–374.
8. **Dorner, A. J., J. P. Stoye, and J. M. Coffin.** 1985. Molecular basis of host range variation in avian retroviruses. J. Virol. **53**:232–233.
9. **Goff, S., P. Traktman, and D. Baltimore.** 1981. Isolation and properties of Moloney murine leukemia virus mutants: use of a rapid assay for release of virion reverse transcriptase. J. Virol. **38**:239–248.
10. **Gospodarowicz, D., J. Moran, D. Brown, and C. Birdwell.** 1976. Clonal growth of bovine vascular endothelial cells: fibroblast growth factor as a survival agent. Proc. Natl. Acad. Sci. USA **73**:4120–4124.
11. **Hanafusa, H.** 1969. Rapid transformation of cells by Rous sarcoma virus. Proc. Natl. Acad. Sci. USA **63**:318–325.
12. **Knutton, S.** 1978. The mechanism of virus induced fusion. Micron. **9**:133–154.
13. **Kohn, A.** 1985. Membrane effects of cytopathogenic viruses. Prog. Med. Virol. **31**:109–167.
14. **Lifson, J. D., M. B. Feinberg, G. R. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moll, F. Wong-Staal, K. S. Steimer, and E. G. Engelman.** 1986. Induction of CD_4 dependent

cell fusion by HTLVIII envelope glycoprotein. Nature (London) **323**:726–728.
15. **Maeda, T., K. Kawasaki, and S. Ohnishi.** 1978. Interaction of influenza virus induced hemolysis and fusion. Proc. Natl. Acad. Sci. USA **78**:4133–4137.
16. **McGowan, J. J., and R. R. Wagner.** 1981. Inhibition of cellular DNA synthesis by vesicular stomatitis virus. J. Virol. **38**: 356–367.
17. **Moss, B.** 1968. Inhibition of HeLa cell protein synthesis by vaccinia virus. J. Virol. **2**:1028–1037.
18. **Nakamura, S., S. Z. Salahuddin, P. Biberfeld, B. Ensoli, P. D. Markham, F. Wong-Staal, and R. C. Gallo.** 1988. Kaposi's sarcoma cells: long-term culture with growth factor from retrovirus infected CD_4^+ T cells. Science **242**:426–430.
19. **Niedt, G. W., and R. A. Schinella.** 1985. Acquired immunodeficiency syndrome. Clinicopathologic study of 56 autopsies. Arch. Pathol. Lab. Med. **109**:727–734.
20. **Rasheed, S., A. A. Gottlieb, and R. F. Garry.** 1986. Cell killing by ultraviolet-inactivated human immunodeficiency virus. Virology **154**:395–400.
21. **Salahuddin, S. Z., S. Nakamura, P. Biberfeld, M. H. Kaplan, P. D. Markham, L. Larsson, and R. C. Gallo.** 1988. Angiogenic properties of Kaposi's sarcoma derived cells after long term culture in vitro. Science **242**:430–433.
22. **Scheid, A., A. L. Caliguiri, R. W. Compans, and P. W. Choppin.** 1972. Isolation of paramyxovirus glycoprotein association of both hemagglutinating and neuroaminidase activity with the larger SV5 glycoprotein. Virology **50**:640–652.
23. **Simon, M. C., W. S. Neckameyer, W. S. Hayward, and R. E. Smith.** 1987. Genetic determinants of neoplastic diseases induced by a subgroup F avian leukosis virus. J. Virol. **61**: 1203–1212.
24. **Sodorski, J., W. C. Goh, C. Rosen, K. Kampbell, and W. A. Haseltine.** 1986. Role of the HTLVIII/LAV envelope in syncytium formation and cytopathogenicity. Nature (London) **322**: 470–474.
25. **Somasundaran, M., and H. L. Robinson.** 1987. A major mechanism of human immunodeficiency virus-induced cell killing does not involve cell fusion. J. Virol. **61**:3114–3119.
26. **Temin, H. M.** 1988. Mechanism of cell killing: cytopathic effect by non-human retroviruses. Rev. Infect. Dis. **10**:399–405.
27. **Weller, S. K., A. E. Joy, and H. M. Temin.** 1980. Correlation between cell killing and massive second-round superinfection by members of some subgroups of avian leukosis virus. J. Virol. **33**:494–506.
28. **Weller, S. K., and H. M. Temin.** 1981. Cell killing by avian leukosis viruses. J. Virol. **39**:713–721.
29. **Wolff, L., and S. Ruscetti.** 1988. The spleen focus-forming virus (SFFV) envelope gene, when introduced into mice in the absence of other SFFV genes, induces acute erythroleukemia. J. Virol. **62**:2158–2163.
30. **Wood, J. G., and F. O. Sarinana.** 1975. The staining of specific nerve glycoproteins on polyacrylamide gels with concanavalin A-peroxidase. Anal. Biochem. **69**:320–322.