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Inhibitors of Retrovirus Infection Are Secreted by Several Hamster Cell Lines and Are Also Present in Hamster Sera

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We have previously shown that Chinese hamster ovary (CHO) cells are resistant to infection by gibbon ape leukemia virus and amphotropic pseudotype retroviral vectors because of the secretion of factors that inhibit retrovirus infection. Such factors were not secreted by any mouse or human cell lines tested. Secretion of the inhibitors and resistance to infection are abrogated by treatment of CHO cells with the glycosylation inhibitor tunicamycin. Here we show that the inhibitory activities against gibbon ape leukemia virus and amphotropic viruses are partially separable and that glycosylation mutations in CHO cells mimic the effects of tunicamycin treatment. We find that several hamster cell lines derived from both Chinese and Syrian hamsters secrete inhibitors of retrovirus infection, showing that these inhibitors are not unique to the CHO cell line. Inhibitory factors are also present in the sera of Chinese and Syrian hamsters but were not detected in bovine serum. These results suggest the presence of specific factors that function to inhibit retrovirus infection in hamsters.

An important mechanism by which animals have evolved resistance to retroviral pathogens involves the germ line acquisition and expression of defective endogenous proviruses to produce a state of resistance to infection by the same and related retroviruses. Resistance to infection is most often due to endogenous expression of viral envelope (Env) proteins that bind to receptors used for virus entry, resulting in an inhibition of receptor export to the cell surface or interference with virus binding to receptors at the cell surface. Examples of such defective endogenous proviruses include the endogenous virus (*ev*) loci that confer resistance to avian leukosis viruses in chickens (1, 33), the endogenous murine virus (*emv*) and *Fv-4* loci that confer resistance to ecotropic murine leukemia viruses in mice (7, 14), and the *Rmcf* locus that confers resistance to mink cell focus-forming virus in mice (3, 17, 34).

Mice carrying the *Fv-4* locus (also known as *Akvr-1*) provide a good example of this phenomenon. The germ line provirus in these animals encodes an apparently intact Env protein but is defective in the production of Gag and Gag-Pol polyproteins because of a large deletion. In addition, virions made from recombinant viruses constructed by using Moloney murine leukemia virus (MoMuLV) *gag-pol* and *Fv-4 env* genes carried *Fv-4* Env protein but were noninfectious, suggesting that the *Fv-4* Env protein is defective and cannot mediate virus entry into cells (24). As a result, the endogenous *Fv-4* provirus cannot directly contribute to endogenous virus production. Synthesis of Env protein from this defective provirus has no apparent deleterious effect in mice and thus represents an ideal strategy for prevention of early events in retrovirus infection. Production of defective proviruses is a frequent event in the normal course of retrovirus infection (37) and would provide the starting material for the selective pressure exerted by pathogenic retroviruses to fix these proviruses in the germ line.

We have identified a factor that is secreted by Chinese hamster ovary (CHO) cells and that blocks infection of these cells by retroviral vectors pseudotyped with amphotropic or gibbon ape leukemia virus (GALV) Env proteins (28). The block to infection and secretion of the inhibitory activity was abolished by treatment of the cells with tunicamycin, an inhibitor of N-linked glycosylation of protein. The block to infection observed in cells that express endogenous Env protein is also relieved by treatment with tunicamycin (32). However, the CHO factor is secreted, unlike normal Env proteins, and we have been unable to complement env^- retroviruses with the CHO factor. These results indicate at least that this inhibitory factor is not an intact Env protein and leave open the possibility that the factor is not an Env protein at all.

Here we show that several hamster cell lines, including normal diploid fibroblasts from Chinese hamsters, are resistant to infection by vectors carrying GALV and amphotropic envelope proteins. In addition, these cell lines secrete factors that inhibit GALV and amphotropic vector infection. Inhibitory factors were also present in serum from both Chinese and Syrian hamsters. These results indicate that these inhibitory factors are not an artifact of the CHO cell line and could represent a biologically important mechanism of retroviral resistance.

MATERIALS AND METHODS

Cells and viruses. CHO-K1 cells (19) were a gift from Carol Jones, Eleanor Roosevelt Institute for Cancer Research, Denver, Colo. CHO cell-derived glycosylation mutants CHO-Lec2 (ATCC CRL 1736) (10, 39) and CHO-Lec8 (ATCC CRL 1737) (9, 38, 39), the $HPRT^-$ Chinese hamster lung cell line E-36 (16), and BHK-21 (ATCC CCL 10) Syrian hamster kidney cells were gifts from Maribeth Eiden, National Institutes of Health, Bethesda, Md. Chinese hamster lung fibroblast cell strains Dede (ATCC CCL 39) and Don (ATCC CCL 16) were obtained from the American Type Culture Collection, Rockville, Md. All hamster cells were maintained in Ham's F12 medium supplemented with 5% fetal bovine serum. HeLa cells (15), NIH 3T3 (TK^-) cells (25), and retrovirus packaging cell lines were maintained in Dulbecco's modified Eagle's medium containing 4.5 g of

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glucose per liter and supplemented with 10% fetal bovine serum.

The MoMuLV-based retroviral vectors LNL (4), LNL6 (4), and LN (27) express the *neo* gene under the transcriptional control of the MoMuLV promoter. These vectors are all functionally identical for the purposes of this study and will be referred to as *neo* vectors. For historical reasons, different *neo* vectors were introduced into the different packaging cell lines used here. Viral pseudotypes were obtained by packaging these *neo* vectors in the following packaging cell lines with the indicated host range: PG13 cells (26), GALV host range; PE501 cells (27), ecotropic host range; and PA317 cells (25), amphotropic host range. Xenotropic pseudotype virus was made by cotransfecting *Mus dunii* tail fibroblasts (6) with cloned NZB xenotropic virus (31) and the LNL *neo* vector and selecting the cells in G418. Virus stocks were made by feeding confluent dishes of virus-producing cells, harvesting the medium 16 to 24 h later, filtering the virus, and storing it at −70°C.

Viral infections. All viral infections were performed in the presence of 4 μg of Polybrene (Sigma) per ml. Unless otherwise indicated, target cells were seeded at 10^5 per 6-cm dish on day 1, fresh medium, virus, and polybrene were added on day 2, and medium containing G418 (1 mg/ml for CHO cells, 1.5 mg/ml for NIH 3T3 cells, and 2 mg/ml for HeLa cells; the concentration indicates the total weight of G418 powder, about half of which is active) was added on day 3. Colonies were stained with Coomassie brilliant blue G (Sigma; 1 g/liter in 40% methanol–10% acetic acid) and counted on day 9. Results are expressed as averages from duplicate dishes from a representative experiment. Values from duplicate dishes generally varied by no more than 20% (the maximum variation was 30%).

CHO cell infections performed after tunicamycin pretreatment were carried out with 0.15 to 0.3 μg of tunicamycin (Boehringer Mannheim, Indianapolis, Ind.) per ml (depending on the batch of tunicamycin). Cells were trypsinized and seeded at 10^5 cells per 6-cm dish. At 4 h after seeding (after cells had become adherent), tunicamycin was added. At 19 h after the addition of tunicamycin, the medium was removed and fresh medium and virus were added.

Conditioned medium was prepared by exposure of culture medium to confluent layers of cells for 24 h. Conditioned medium could be added immediately before, during, or immediately after the addition of virus in tunicamycin-treated and untreated cell infection assays with similar effects.

RESULTS

CHO cells secrete inhibitors of retrovirus infection that are partially separable by size into two fractions. We have previously shown that culture medium conditioned by CHO cells contains protein factors that inhibit CHO cell infection by retroviral vectors pseudotyped with either amphotropic or GALV Env proteins (28). Since these two viruses use different receptors for entry into cells, it was difficult to explain the result on the basis of the secretion of a single factor. We therefore tried to resolve the two inhibitory activities by gel filtration chromatography.

Serum-free medium conditioned for 24 h by confluent layers of CHO cells was treated with 80% ammonium sulfate to precipitate the inhibitory activity (28). This precipitate was resuspended in 1 ml of serum-free medium and fractionated over a Sephadex G-100 gel filtration column. Fractions obtained from the column were tested for the ability to

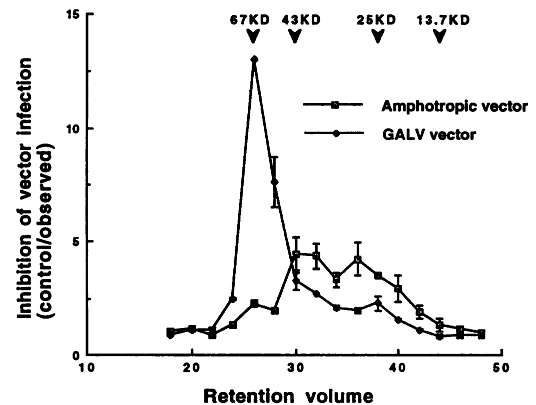

FIG. 1. Size fractionation of inhibitors of GALV and amphotropic vector infection of CHO cells. Factors that inhibit retrovirus infection were concentrated from serum-free medium conditioned by CHO cells for 24 h by precipitation with 80% ammonium sulfate and were fractionated over a Sephadex G-100 gel filtration column as previously described (28). Samples of each 2-ml column fraction from the same column run were assayed for the ability to inhibit amphotropic or GALV pseudotype *neo* vector infection of tunicamycin-treated CHO cells. Samples of 200 μl were assayed for inhibition of GALV vector infection, and 50-μl samples were assayed for inhibition of amphotropic vector infection to compensate for the higher sensitivity of amphotropic vectors to the inhibitory factors. Inhibition of vector infection was calculated by dividing the infection rate (CFU per milliliter) observed in the absence of column fraction addition by that observed in the presence of the column fraction addition. Bars indicate standard deviations of duplicate samples, and the positions of protein size markers are shown at the top of the figure. Results from one column fractionation experiment are shown; a second experiment gave similar results.

inhibit retrovirus infection of CHO cells treated with the glycosylation inhibitor tunicamycin. Tunicamycin pretreatment renders CHO cells susceptible to amphotropic vector infection and improves the infection rate of GALV-pseudotyped vectors; therefore, all assays were performed by using tunicamycin-treated CHO cells as targets for vector infection. Inhibition of infection was observed for a retroviral vector pseudotyped with either GALV or amphotropic Env proteins as described previously (28). These two inhibitory activities were partially separable by gel filtration chromatography (Fig. 1). The peak of inhibitory activity for vectors pseudotyped with amphotropic Env corresponded to a molecular mass of 20 to 45 kDa. However, when the same column fractions were assayed for inhibition of a vector pseudotyped with GALV env, the inhibitory activity was present in a different set of fractions corresponding to a molecular mass of 45 to 70 kDa. These results suggest that two or more secreted proteins are involved in the inhibition of GALV and amphotropic vector infection of CHO cells and presumably explain why two different viruses that use different receptors for cell entry are affected.

Other hamster cell lines also secrete inhibitors of retrovirus infection. To determine whether the secretion of inhibitors of retrovirus infection is unique to CHO cells, we analyzed several other independently isolated hamster cell lines. Dede and Don cells are diploid lung fibroblast cells isolated from female and male adult Chinese hamsters, respectively. The E-36 cell line was derived from the V79 lung fibroblast cell line (ATCC CCL 93) isolated from a male Chinese hamster. BHK is a diploid kidney cell line derived from a male Syrian

TABLE 1. Several hamster cell lines are resistant to retrovirus infection and respond to inhibitors of retrovirus infection secreted by CHO cells[a]

Targets cell	Amphotropic vector titer[b] (CFU/ml) with conditioned medium:		Inhibition (%)[c]	GALV vector titer[b] (CFU/ml) with conditioned medium:		Inhibition (%)[c]
	Absent	Present		Absent	Present	
Dede	<1			8.5×10^2	2.0×10^2	64 ± 16
Don	24	1.5	88 ± 7	1.0×10^4	5.4×10^3	53 ± 12
E-36	68	6.0	87 ± 5	3.4×10^4	1.5×10^4	58 ± 3
BHK	<2			<5		
CHO	<1			8.7×10^2	15	95 ± 5
HeLa				3×10^5		
NIH 3T3	5×10^6					

[a] Target cells were infected with amphotropic or GALV pseudotype *neo* vectors in the presence or absence of 1 ml of conditioned medium from CHO cells, and G418-resistant colony formation was measured.

[b] Vector titers are averages of duplicate dishes in a representative experiment.

[c] Values for inhibition of infection are means and standard deviations for two or three independent experiments.

hamster. These cell lines were tested for their susceptibility to vector infection, assayed for sensitivity to the factors secreted by CHO cells, and screened for production of activities which inhibit retroviral infection.

The hamster cells were resistant to infection by vectors pseudotyped with ecotropic, xenotropic, amphotropic, and GALV Env proteins (Table 1; data not shown). A dramatic resistance to amphotropic vector infection was observed in comparison with infection rates in susceptible NIH 3T3 cells (Table 1, first data column), whereas infection by GALV pseudotype vector was less severely affected in comparison with infection rates in susceptible HeLa cells (Table 1, fourth data column). Tunicamycin treatment increased the susceptibility of Dede, Don, and E-36 cells to vectors pseudotyped with amphotropic Env and increased the susceptibility of Dede and Don cells to vectors pseudotyped with GALV Env (data not shown). Tunicamycin treatment had no effect on GALV vector infection of E-36 cells, presumably because E-36 cells were already relatively susceptible to GALV vector infection (data not shown). This pattern of resistance to retrovirus infection, which can be abrogated by exposure of the cells to tunicamycin, is similar to results previously obtained with CHO cells (28).

Infection of hamster cells with vectors pseudotyped by amphotropic or GALV Env could be inhibited by addition of medium conditioned by CHO cells (Table 1). We observed about a 10-fold decrease for the amphotropic vector in Don and E-36 cells and a 2- to 3-fold decrease for the GALV vector in Dede, Don, and E-36 cells. The amphotropic vector did not infect Dede and BHK cells, and the GALV vector did not infect BHK cells. These results show that Dede, Don, and E-36 hamster cells respond to factors secreted by CHO cells which inhibit GALV vector infection and that Don and E-36 cells respond to factors which inhibit amphotropic vector infection.

We next tested conditioned medium from Dede, Don, E-36, and BHK hamster cells for the presence of a factor(s) that could inhibit retrovirus infection of CHO cells (Table 2). All of these hamster cell lines secreted inhibitors of amphotropic vector infection. Dede, Don, and BHK cells secreted inhibitors of GALV vector infection, but the E-36 cells did

TABLE 2. Several hamster cell lines secrete factors that inhibit retrovirus infection of CHO cells[a]

Source of conditioned medium	Amphotropic *neo* vector titer (CFU/ml)[b]	Inhibition (%)	GALV *neo* vector titer (CFU/ml)	Inhibition (%)
None	340		3×10^3	
Dede	<10	>97	2×10^2	93
Don	<10	>97	1×10^2	97
E-36	50	85	4×10^3	
BHK	115	66	1.5×10^3	50
CHO	<10	>97	60	98

[a] CHO cells were infected with amphotropic or GALV pseudotype *neo* vectors in the presence or absence of 1 ml of medium conditioned by the indicated cells for 24 h, and G418-resistant colony formation was measured. Results are average values from duplicate dishes in a representative experiment.

[b] For measurement of amphotropic vector titer, the CHO cells were treated with 0.15 μg of tunicamycin per ml for 19 h prior to infection.

not secrete such inhibitors. These results indicate that normal diploid low-passage hamster lung fibroblasts can secrete retrovirus infection inhibitors with activities similar to those secreted from CHO cells. Thus the secretion of factors that inhibit retrovirus infection is not a unique property of CHO cells, perhaps because of the long passage history or transformed phenotype of these cells.

Mutations in the glycosylation pathway of CHO cells increase their susceptibility to retroviral infection. CHO cells become susceptible to infection by most murine retroviruses after treatment with the glycosylation inhibitor tunicamycin and no longer secrete inhibitors of retrovirus infection, suggesting that glycosylation of the inhibitors of infection is necessary for their secretion or for their inhibitory activity (28). To better establish the role of glycosylation in the production of the inhibitory factors, we examined retrovirus infection in two glycosylation mutants derived from CHO cells. These CHO cell mutants have been selected for the ability to grow in the presence of the plant lectin wheat germ agglutinin (39), and they have decreased ability to transport precursors of protein glycosylation (CMP-sialic acid [CHO-Lec2] [10] or UDP-galactose [CHO-Lec8] [9]) into Golgi vesicles. The Lec8 mutation is more pleiotropic because incorporation of galactose is necessary for protein sialylation.

Both CHO-Lec2 and Lec8 glycosylation mutants were much more susceptible to infection by vectors carrying a variety of Env proteins than were the parental CHO cells (Table 3). The CHO-Lec8 cells were more susceptible to

TABLE 3. Mutations that inhibit glycosylation in CHO cells relieve the block to retroviral infection[a]

Vector pseudotype	Vector titer (CFU/ml) on:		
	CHO-Lec2	CHO-Lec8	CHO
GALV	1×10^6	1×10^6	3×10^3
Amphotropic	4×10^4	3×10^5	<1
Ecotropic	50[b]	5×10^4	<1
Xenotropic	2×10^4	1×10^5	1

[a] Target cells were infected with *neo* vectors having the indicated pseudotype, and colony formation was measured. Results are average values from duplicate dishes in a representative experiment.

[b] The vector has an apparent titer of 9×10^4 CFU/ml when assayed on CHO-Lec2 cells treated for 19 h prior to infection with 0.15 μg of tunicamycin per ml.

TABLE 4. Assay of CHO glycosylation mutants for production of inhibitors of retrovirus infection[a]

Source of conditioned medium	Cells used to assay inhibitory activity	Vector pseudotype	Vector titer (CFU/ml)[b]	Inhibition (%)[c]
None	CHO	GALV	5,000	
CHO-Lec8	CHO	GALV	5,000	2 ± 3
CHO-Lec2	CHO	GALV	4,000	12 ± 11
CHO	CHO	GALV	200	90 ± 9
None	CHO + Tun[d]	Amphotropic	440	
CHO-Lec8	CHO + Tun	Amphotropic	300	31 ± 3
CHO-Lec2	CHO + Tun	Amphotropic	110	72 ± 4
CHO	CHO + Tun	Amphotropic	5	99.2 ± 0.5

[a] CHO cells were infected with amphotropic or GALV pseudotype *neo* vectors in the presence or absence of 1 ml of medium conditioned by the indicated cells for 24 h, and G418-resistant colony formation was measured.
[b] Vector titer values are averages of duplicate dishes in a representative experiment.
[c] Values for inhibition of infection are means and standard deviations for two independent experiments.
[d] Tun indicates that the CHO cells were treated with tunicamycin prior to infection.

infection than the CHO-Lec2 cells were, especially for the ecotropic vector, in congruence with the more comprehensive glycosylation mutation present in the CHO-Lec8 cells. Treatment of the CHO-Lec2 cells with tunicamycin before ecotropic vector infection increased the apparent titer of the vector to 9×10^4 CFU/ml (data not shown), indicating that the remaining inhibition of infection observed is due to residual glycosylation in the cells and not to some unrelated blockage to ecotropic vector infection. In summary, mutations in the glycosylation pathway of CHO cells render the cells more susceptible to retrovirus infection, an effect similar to that observed with the glycosylation inhibitor tunicamycin.

CHO glycosylation mutants do not secrete an inhibitor of GALV vector infection, and they secrete reduced levels of inhibitors of amphotropic vector infection. We tested the ability of medium conditioned by CHO-Lec8 and CHO-Lec2 cells to inhibit the infection of CHO cells by amphotropic or GALV pseudotype vectors (Table 4). As expected from their high susceptibility to GALV vector infection, neither CHO-Lec2 or CHO-Lec8 cells produced an activity which inhibited infection of CHO cells by the GALV vector, whereas medium from CHO cells inhibited infection by 10-fold. Likewise, medium conditioned by CHO-Lec8 cells had little effect on amphotropic vector infection of tunicamycin-treated CHO cells, whereas medium from CHO cells inhibited infection by 99%. Medium conditioned by CHO-Lec2 cells inhibited amphotropic vector infection of tunicamycin-treated CHO cells by 72%. As with ecotropic vector infection, the ability of CHO-Lec2 cells to partially glycosylate protein has presumably allowed secretion of small amounts of active inhibitor into medium conditioned by these cells.

Infection of CHO-Lec2 and CHO-Lec8 cells is inhibited by conditioned medium from CHO cells. To distinguish between reduced production of inhibitory factor(s) by the CHO glycosylation mutants and the inability of the mutants to respond to the inhibitory proteins, we tested the sensitivity of the CHO mutants to inhibitory factors secreted by wild-type CHO cells. Infection of CHO-Lec2 or CHO-Lec8 cells with vectors pseudotyped with amphotropic Env was inhibited substantially, about fivefold on average (Table 5). Infection with a GALV-pseudotype vector was inhibited by about twofold. We observed a less-than-twofold inhibitory effect on xenotropic vector infection and essentially no effect on ecotropic vector infection. We hypothesize that the reduced ability of CHO cell-conditioned medium to inhibit infection of the CHO-Lec mutants (Table 5) in comparison with CHO cells (Table 4) is due to very low levels of inhibitory factor production by the CHO-Lec mutants (Table 4), which would supplement the activity of exogenous factors added to the infection assay. These results show that the CHO-Lec mutants are still sensitive to inhibitors of amphotropic and GALV vector infection.

Sera from Chinese and Syrian hamsters inhibit amphotropic vector infection of tunicamycin-treated CHO cells. On the basis of the results that several hamster cell lines derived from different hamster tissues secrete inhibitors of retrovirus infection, we tested hamster sera for similar inhibitory activities. Both Chinese and Syrian hamster sera inhibited amphotropic vector infection of tunicamycin-treated CHO cells but had no effect on GALV or ecotropic vector infection of CHO cells with or without tunicamycin pretreatment (Table 6). The inhibitory effect on amphotropic vector infection was dose dependent (shown for Syrian hamster serum in Fig. 2). Inhibition of amphotropic vector infection was not due to nonspecific toxicity of the serum against CHO cells, since both GALV and ecotropic infection rates were unaffected by the presence of hamster serum. Amphotropic and GALV vector infection of HeLa cells was not significantly affected by the presence of hamster sera; therefore, the inhibitor of amphotropic vector infection apparently does not interact directly with virions to inhibit infection. These

TABLE 5. Infection of CHO-Lec2 and CHO-Lec8 cells is inhibited by the addition of medium conditioned by CHO cells[a]

Vector pseudotype	Vector titer (CFU/ml) on CHO-Lec2[b] with conditioned medium:		Inhibition (%)[c]	Vector titer (CFU/ml) on CHO-Lec8[b] with conditioned medium:		Inhibition (%)[c]
	Absent	Present		Absent	Present	
GALV	2×10^5	9×10^4	46 ± 12	3×10^5	1×10^5	50 ± 14
Amphotropic	3×10^4	4×10^3	77 ± 13	1×10^5	1×10^4	78 ± 14
Ecotropic	NA[d]	NA	NA	5×10^4	5×10^4	10 ± 6
Xenotropic	2×10^4	9×10^3	47 ± 7	1×10^5	7×10^4	25 ± 19

[a] CHO-Lec2 or CHO-Lec8 cells were infected with *neo* vectors having the indicated pseudotypes in the presence or absence of 1 ml of medium conditioned by CHO cells, and G418-resistant colony formation was measured.
[b] Vector titers are averages of duplicate dishes in a representative experiment.
[c] Values for inhibition of infection are means and standard deviations for three independent experiments.
[d] NA, not applicable (CHO-Lec2 cells are infected very poorly with vectors having an ecotropic pseudotype).

TABLE 6. Hamster sera inhibit amphotropic but not GALV pseudotype *neo* vector infection of tunicamycin-treated CHO cells[a]

Additional serum	Cells used to assay inhibitory activity	Vector pseudotype	Vector titer (CFU/ml)[b] with additional serum: Absent	Present	Inhibition (%)[c]
Syrian hamster serum	CHO	GALV	1×10^4	1×10^4	
	CHO + Tun[d]	GALV	5×10^5	5×10^5	
	CHO + Tun	Amphotropic	6×10^2	<10	>98
	CHO + Tun	Ecotropic	4×10^4	4×10^4	
	HeLa	GALV	3×10^5	5×10^5	
	HeLa	Amphotropic	3×10^5	3×10^5	
Chinese hamster serum	CHO	GALV	1×10^4	1×10^4	
	CHO + Tun	GALV	3×10^5	3×10^5	
	CHO + Tun	Amphotropic	1×10^3	15	>98
	CHO + Tun	Ecotropic	2×10^4	2×10^4	
	HeLa	GALV	3×10^5	6×10^5	
	HeLa	Amphotropic	3×10^5	2×10^5	
Fetal bovine serum	CHO + Tun	Amphotropic	1×10^3	2×10^3	

[a] The indicated target cells were infected with amphotropic or GALV pseudotype *neo* vectors in culture medium containing 5% fetal bovine serum (additional serum absent) or 5% fetal bovine serum plus 12.5% Syrian hamster serum, 5% fetal bovine serum plus 5% Chinese hamster serum, or 30% fetal bovine serum (additional serum present), and G418-resistant colony formation was measured.
[b] Results are average values from duplicate assays.
[c] Inhibition is reported only when over 50%.
[d] Tun indicates that the cells were treated with tunicamycin prior to infection.

results show that Chinese and Syrian hamsters produce a factor(s) that inhibits hamster cell infection by amphotropic retroviruses and that may be related to the activity secreted by cultured hamster cells.

DISCUSSION

We have shown here that several hamster cell lines secrete factors that inhibit GALV and amphotropic retrovirus infection of hamster cells and that hamster serum contains an inhibitor of amphotropic retrovirus infection. The most likely identity of the retroviral infection inhibitors described here is that they are retroviral Env proteins synthesized from endogenous hamster proviruses. Secretion of these inhibitors by cultured hamster cells and their appearance in the sera of hamsters could be due to dissociation or cleavage of the amino terminus (SU) from the membrane-anchoring carboxy terminus (TM) of the Env protein or to the synthesis of a truncated Env protein that is secreted freely because of lack of a carboxy-terminal membrane anchor. Env antigens can be detected in the sera of mice (11, 20, 40) and are secreted by mouse cells in culture (5, 13), and purified SU protein of a mouse virus (gp71 from Friend murine leukemia virus) can inhibit retrovirus infection of mouse cells (35), making this hypothesis plausible. However, our results suggest at least that these inhibitory factors are not functional Env proteins, since introduction of a retroviral Gag-Pol expression construct and a retroviral vector into hamster cells did not result in production of infectious virions (28). In addition, it is surprising that the inhibitory factors described here block only infection of hamster cells and not infection of mouse or human cells, whereas the normal Env proteins of the viruses that are blocked (amphotropic and GALV pseudotype viruses) allow virus binding to and infection of a wide range of mammalian and avian cells (26).

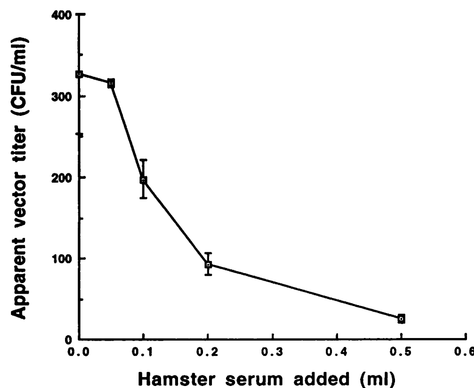

FIG. 2. Addition of Syrian hamster serum inhibits amphotropic *neo* vector infection of tunicamycin-treated CHO cells. Tunicamycin-pretreated CHO cells were infected with amphotropic *neo* virus in the presence of various amounts of Syrian hamster serum, and G418-resistant colony formation was measured. Results are average values from duplicate dishes in a representative experiment.

Human serum can lyse retrovirus virions in an antibody-independent manner (41) that is initiated by complement component C1q binding to the p15E portion of the retroviral Env protein (2, 8). Retroviruses are also lysed by ape, monkey, and cat sera but not by calf, sheep, swine, guinea pig, rat, mouse, or chicken sera (36, 42). Complement-mediated lysis of all retrovirus pseudotypes used here, including ecotropic, xenotropic, amphotropic, and GALV pseudotypes, has been documented (2, 8, 36, 41, 42). However, we can rule out a role for hamster complement in the experiments described here. The inhibitory activities present in hamster sera inhibit retrovirus infection only of hamster cells but not human cells (Table 6). If complement-mediated lysis of retrovirus virions were involved, infection rates in both cell types should be decreased. In addition, our previous work showed a similar result for the inhibitory activity secreted by CHO cells, which inhibited infection only of hamster cells but not infection of mouse or human cells (28). Therefore, hamster complement is not responsible for the inhibition of retrovirus infection that we observe. Although

we have not directly shown the presence of active complement in our hamster sera, the sera were prepared in a manner that should preserve complement activity. Therefore, it is likely that hamster complement does not directly lyse the retroviruses used here, similar to the results obtained with sera from other closely related rodents such as rats and mice (36, 42).

An inhibitor of retrovirus infection is produced by cells cultivated from New Zealand Black (NZB) mice (21, 22). Production of the NZB inhibitor is related to the production of xenotropic virus from these cells, because the inhibitor is not produced by uninfected mouse cells but is produced by human cells infected with xenotropic virus. This inhibitor of retrovirus infection has some of the properties associated with the inhibitors described here but appears to act after virus penetration, perhaps at the level of reverse transcription of viral RNA (21). In contrast, the virus infection inhibitors described here act at the level of virus penetration mediated by specific Env proteins. For example, a dramatic inhibitory effect on virus infection of CHO cells is observed for vectors with GALV or amphotropic Env proteins, but little inhibition of CHO cell infection by otherwise identical vectors with ecotropic or xenotropic Env surface proteins is observed (28).

Other inhibitors of retrovirus infection that appear to neutralize virions directly have been found in the sera of mice. These include a factor named oncornavirus-inactivating factor, which has an apparent molecular mass of 10,000 kDa (12, 29, 30) and another factor with an apparent molecular mass of less than 30 kDa (23). These factors appear unrelated to the inhibitors described here that do not have a direct effect on virions (28), and do not appear to be related to retroviral Env proteins.

Although results presented here for experiments with hamsters and previous results with mice suggest a protective effect of endogenous expression of inhibitors of retrovirus infection, in general it is not clear whether these effects are cell autonomous or can extend to other cells in animals that do not express the inhibitor. In this regard, irradiated $Fv\text{-}4^+$ mice receiving transplants of mixtures of marrow from $Fv\text{-}4^+$ (resistant) and $Fv\text{-}4^-$ (sensitive) mice were susceptible to Friend MuLV-induced disease when as little as 10% of the transplanted cells were from $Fv\text{-}4^-$ mice (18). Presumably, the virus replicates and causes disease only in the $Fv\text{-}4^-$ marrow cells. This experiment shows that an excess of cells expressing the *Fv-4* locus does not protect cells in the same animal that do not express the locus, arguing for the cell-autonomous nature of the inhibition to retrovirus infection. In contrast, our data argue that the inhibitors of retrovirus infection from hamsters should act in *trans* to protect cells that cannot make the factor, and they suggest a more effective strategy for blocking retrovirus infection.

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