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Type C Retrovirus Inactivation by Human Complement Is Determined by both the Viral Genome and the Producer Cell

YASUHIRO TAKEUCHI,[1,2] FRANÇOIS-LOÏ C COSSET,[1] PETER J. LACHMANN,[3] HIDECHIKA OKADA,[4] ROBIN A. WEISS,[1] AND MARY K. L. COLLINS[1]*

Chester Beatty Laboratories, Institute of Cancer Research, London SW3 6JB,[1] and Molecular Immunopathology Unit, MRC Centre, Cambridge CB2 2QH,[3] United Kingdom, and Department of Hygiene, Gunma University School of Medicine, 3-39-22 Showa-machi, Maebashi 371,[2] and Department of Molecular Biology, Nagoya City University School of Medicine, Nagoya 467,[4] Japan

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The inactivation of type C retroviruses by human serum may be a considerable impediment to the use of retroviral vectors in vivo for gene therapy. Here we show that virus inactivation is dependent both on the virus and on the cell line used to produce the virus. All viruses produced from murine NIH 3T3 or dog Cf2ThS+L− cells are sensitive to human serum. In contrast, those produced from mink Mv-1-Lu and human HOS or TE671 cells are at least partially resistant, with the exception of murine leukemia viruses. In particular, the feline endogenous virus RD114 is completely resistant to a panel of eight human sera when produced from Mv-1-Lu or HOS cells. This differential resistance is controlled by the viral envelope proteins. Virus inactivation can be correlated with the ability of the producer cells to be lysed by human serum. Inactivation of sensitive viruses requires the classical pathway of complement but does not require virion lysis.

The inhibition of the infectivity of type C retroviruses by human serum was first demonstrated nearly 20 years ago. Welsh and colleagues (30, 31) reported that four strains of murine leukemia viruses (MLVs), and Moloney sarcoma virus pseudotypes with the envelope specificity of gibbon ape leukemia virus (GALV) or simian sarcoma-associated virus (SSAV), were inactivated by fresh but not heated human serum. Lysis of these viruses, feline leukemia virus (FeLV), cat endogenous virus RD114, SSAV, baboon endogenous virus (BaEV) M28, and the type D virus Mason-Pfizer monkey virus by human serum was demonstrated by the release of reverse transcriptase (RT) activity from virions (22, 30, 31). Complement-depleted or -deficient human sera failed to cause viral lysis, and complement consumption was observed when viruses were added to human serum (30). MLVs were then shown to be lysed following direct, antibody-independent triggering of the human classical complement pathway mediated by the binding of the C1q component to MLV virions (5). An isolated 15-kDa virion protein with a pI of 7.5, proposed to be the p15E transmembrane (TM) protein, was shown to trigger complement (3).

It was argued that these observations might explain the ease of isolation of RNA tumor viruses and other type C retroviruses from mice, cats, and chickens but not from humans (30). Indeed, the subsequently discovered human retroviruses human T-cell leukemia virus type I and human immunodeficiency virus type 1 were both found to be resistant to inactivation by human serum (2, 9). Furthermore, murine serum failed to inactivate murine, or any other, type C retroviruses (22, 30, 31), and chicken serum failed to lyse avian myeloblastosis virus or several viruses from other species (22, 30). However, in several species, sera could lyse viruses naturally infectious for that species; for example, cat sera lysed FeLV, baboon sera lysed BaEV-M28, and rhesus monkey sera lysed Mason-Pfizer monkey virus (22, 31). No specificity in viral lysis was observed in such studies; sera from species capable of lysis lysed all type C and D retroviruses tested (22). Thus, no clear correlation could be made between either the release of endogenous type C retroviruses or horizontal retroviral transmission and resistance of retroviruses to host complement lysis.

Retroviruses are currently being used as delivery vehicles in a number of human gene therapy trials (15). In some applications, direct gene delivery to cells in vivo, for example, the delivery of the herpes simplex virus thymidine kinase gene by recombinant retroviruses to tumors (18), is being attempted. For these trials, MLV amphotropic strain (MLV-A) packaging cells, constructed in murine NIH 3T3 cells, are being used to produce recombinant retroviruses which are sensitive to human complement inactivation. Therefore, to develop retroviruses resistant to lysis by human serum which will be more efficient for in vivo gene delivery applications, we examined the mechanisms which control retroviral inactivation by human serum. In this report, we demonstrate that both viral and producer cell components determine the sensitivity of retroviral vectors to human complement. Viral lysis is unnecessary for viral inactivation. Virus-producer cell combinations which produce virions resistant to prolonged exposure to a panel of human sera are identified. These data will allow the construction of recombinant retroviral packaging cells capable of producing complement-resistant virions, which will be suitable for applications of gene therapy that require in vivo gene delivery.

MATERIALS AND METHODS

Cells. Murine NIH 3T3 and PG13 (16), GP+EAM12 (13), and GP+E86 (14) packaging cell lines were cultivated in Dulbecco modified Eagle's medium (DMEM) supplemented with 10% newborn calf serum. Mink Mv-1-Lu, dog Cf2ThS+L−, human HOS, and human TE671 cells (23, 26) were

* Corresponding author. Mailing address: Chester Beatty Laboratories, Institute of Cancer Research, 237 Fulham Rd., London SW3 6JB, United Kingdom. Phone: 44-71-352-8133. Fax: 44-71-352-3299.

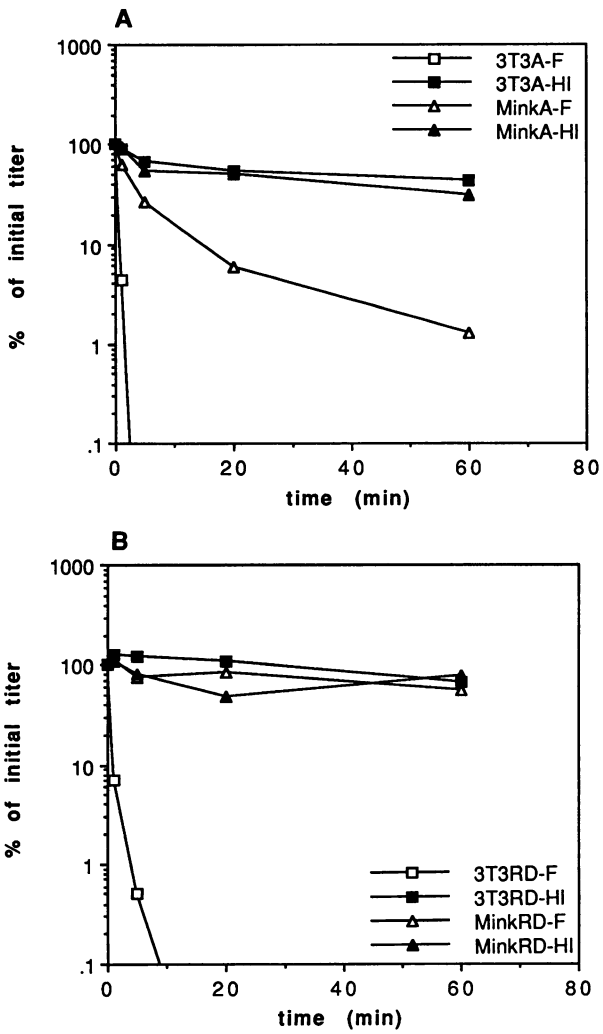

FIG. 1. Time course of virus inactivation by fresh human serum. LacZ(MLV-A) from NIH 3T3 cells (3T3A) and Mv-1-Lu cells (MinkA) (A) and LacZ(RD114) from NIH 3T3 cells (3T3RD) and Mv-1-Lu cells (MinkRD) (B) were incubated with either fresh (F) or heat-inactivated (HI) normal human serum (NHS-2). The virus-serum mixture was plated on TE671 cells at various time points.

cultivated in DMEM supplemented with 10% fetal calf serum (FCS).

Viruses. The MFGnlslacZ genome was introduced into NIH 3T3, Mv-1-Lu, Cf2ThS+L−, HOS, and TE671 cells by infection with LacZ(MLV-A) produced from the ΨCRIP packaging line as previously described (26). After cell cloning by limiting dilution, clones which gave a high titer of *lacZ* pseudotype in a pilot rescue experiment were selected. *lacZ* pseudotypes containing helper virus were produced by infection of these cell clones with replication-competent MLV-A 1504 strain, MLV-XNZB, RD114, BaEV-M7, SSAV, GALV-SF, and FeLV-B as previously described (23, 26). Viruses were harvested either in DMEM–10% FCS or serum-free Opti-MEM (Gibco-BRL, United Kingdom) for experiments using complement-deficient sera, filtered through a 0.45-μm-pore-size filter, and frozen at −70°C until use. LacZ(RD114) was obtained from NIH 3T3 cells by transfection with full-length proviral DNA (sc3c; kindly supplied by S. O'Brien). Helper-free pseudotypes from NIH 3T3 cells were obtained from PG13, GP+EAM12, and GP+E86 packaging lines by either transduction with helper-free LacZ(MLV-A) or transfection of MFGnlslacZ. Helper-free pseudotypes from Mv-1-Lu cells were obtained by transfection of MFGnlslacZ Mv-1-Lu cells with separate expression plasmids encoding MLV *gag* and *pol* genes and the MLV-E, MLV-A, or RD114 envelope gene. All virus stocks had original *lacZ* titers ranging from 2×10^4 to 4×10^6 on appropriate assay cells.

Serum sources. Human sera were collected from eight healthy subjects, one C2-deficient patient, two C7-deficient patients, and one C9-deficient patient, aliquoted, and frozen at −70°C until use. Heat inactivation was carried out at 56°C for 1 h. C3-depleted serum was obtained by treatment with 25 μg of Sepharose-CL-4-bound cobra venom factor per ml of serum at 37°C overnight. C4-depleted serum was prepared by treating 1 part normal serum with 2 parts C1 at 37°C for 1 h.

Infection. Forty microliters of virus dilution was mixed with an equal volume of fresh human serum, heat-inactivated human serum, or heat-inactivated FCS in 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (pH 7) with less than 2% FCS from virus harvest and incubated at 37°C for up to 1 h. After incubation, the virus-serum mixture was diluted with 1 ml of DMEM with 8 μg of Polybrene per ml and plated on the assay cells in 24-well plates. MLV-E was assayed on NIH 3T3 cells, MLV-A, MLV-X, BaEV, and RD114 were assayed on Mv-1-Lu or TE671 cells, and GALV, SSAV, and FeLV-B were assayed on TE671 cells. Assay cells were seeded at 5×10^4 cells per well in 24-well plates on the day before infection. For experiments using complement-deficient sera, cells were washed extensively in Opti-MEM before infection. After 4 h of infection, virus was removed and cells were cultivated in growth medium. Two days after infection, cells were stained with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) in situ, and *lacZ*-positive colonies were counted as previously described (26). Relative titers (percent) for fresh and heat-inactivated human serum treatment versus FCS treatment are shown.

Cr release. Cells (2×10^6) were removed from plates with EDTA, washed, and resuspended in 200 μl of sodium [^{51}Cr]chromate (1 mCi/ml; Amersham) at 37°C for 1 h. After labeling, cells were washed and resuspended in DMEM with 10% FCS. After incubation at 37°C for 30 min, cells were collected by centrifugation, washed with serum-free DMEM, and resuspended in serum-free DMEM at 2×10^5/ml. Fifty microliters of cell suspension was mixed with 100 μl of serum dilution in a V-bottom microtiter well. Plates were incubated at 37°C for 1 h, and the percent specific ^{51}Cr released into cell-free supernatant was determined by the following formula: (release with serum − release with serum-free medium)/(release with 1% Nonidet P-40 − release with serum-free medium) × 100.

RT release. Twelve milliliters of cell supernatant was harvested from confluent producer cells in serum-free Opti-MEM and clarified by low-speed centrifugation and by filtration through 0.45-μm-pore-size filters. Virus was concentrated by ultracentrifugation (12,000 × *g*, 1 h, 4°C). The viral pellet was then suspended in 120 μl of cold Opti-MEM and aliquoted in four tubes (30 μl in each); 30 μl of 0.5% Triton X-100, FCS, heat-inactivated human serum, or fresh human serum was added, and the tubes were incubated for 1 h at 37°C. RT activity was measured as previously described (7). Eighty microliters of RT mix containing 5 μl of Tris (1 M, pH 8.0), 5 μl of dithiothreitol (0.1 M), 2.5 μl of $MnCl_2$ (0.04 M), 10 μl of KCl (1 M), 1 μl of primer-template [1 mg of poly(rA)·$p(dT)_{12-18}$ (Pharmacia) per ml], 31.5 μl of H_2O, and 20 μl of

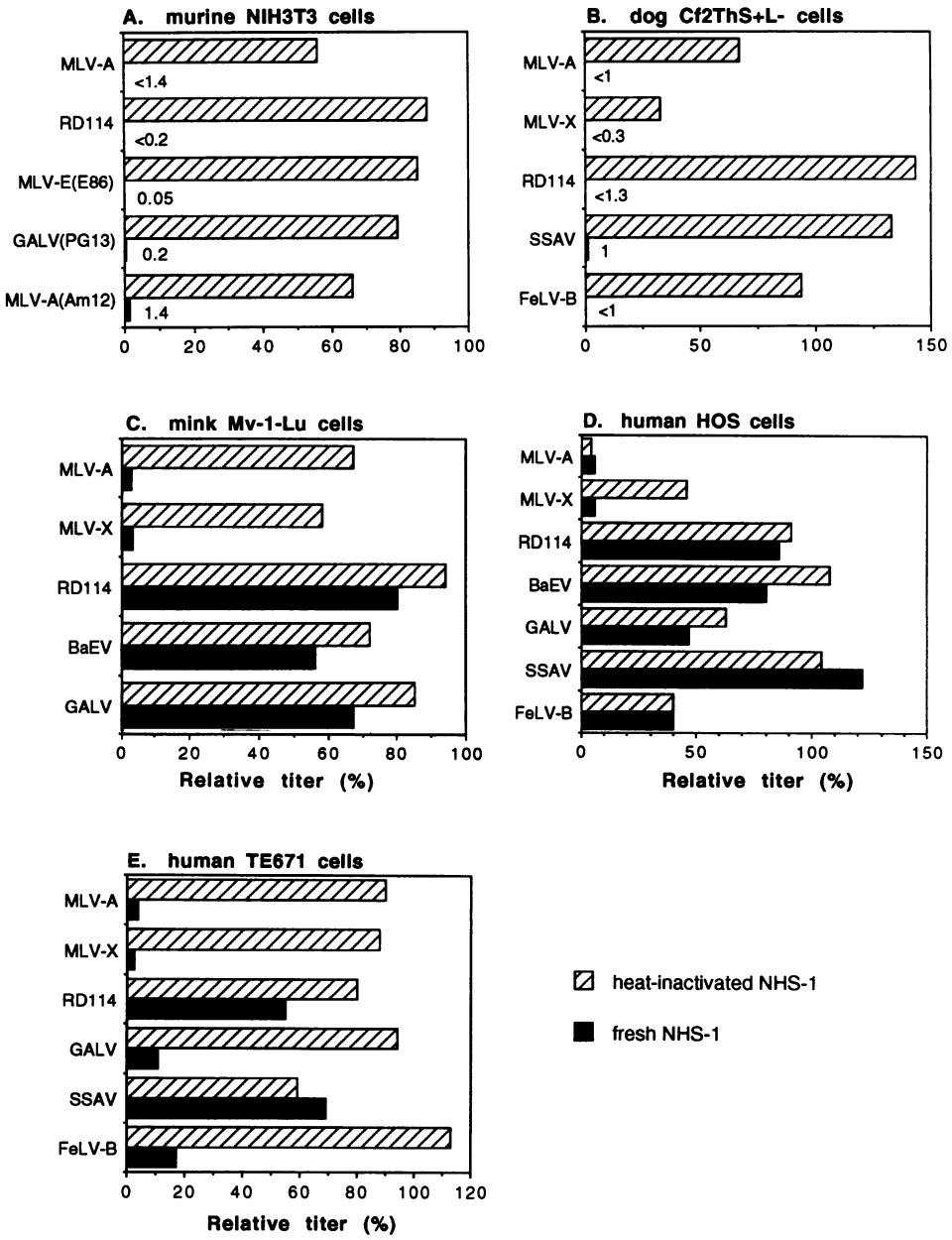

FIG. 2. Variable sensitivity to human serum of viruses produced from different cell lines. Viruses produced from NIH 3T3 cells including packaging cell lines PG13, GP+EAM12 (Am12), and GP+E86 (E86) (A), Cf2ThS+L− cells (B), Mv-1-Lu cells (C), HOS cells (D), and TE671 cells (E) were examined. Surviving titers of *lacZ* pseudotypes after incubation with fresh and heat-inactivated human serum (NHS-1) and FCS in a 1:1 mixture at 37°C for 1 h were measured. Relative titers for fresh and heat-inactivated human serum treatment versus FCS treatment are shown.

[^{3}H]TTP (0.1 mCi of [*methyl*-^{3}H]TTP [Amersham] per ml) and 20 μl of virus-serum mixture were added to wells of a 96-well plate. Duplicate reaction mixtures were incubated at 37°C. Polymerized TTP was separated from free TTP on DE81 filter mats prewetted with 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), using a cell harvester. Filters were washed for 30 s with 2× SSC and dried, and each spot was counted. Percent specific RT released by serum was estimated by the following formula: (release by serum − release by FCS)/(release by Triton X-100 − release by FCS) × 100.

RESULTS

MLV-based retroviral vectors encoding a marker gene can be efficiently packaged by a variety of type C retroviruses (27). These retroviral pseudotypes provide a rapid, quantitative assay for infectivity of the rescuing virus. We therefore used pseudotypes carrying an MLV vector encoding β-galactosidase (26) to assess the sensitivity of type C retroviral infectivity to inactivation by human sera. Figure 1A shows the result of incubation of LacZ(MLV-A), produced from murine NIH 3T3 cells or mink Mv-1-Lu cells, for various times with human

TABLE 1. Summary of virus inactivation by human serum

Virus	Virus inactivation in indicated producer cells[a]				
	Murine NIH 3T3	Dog Cf2ThS+L−	Mink Mv-1-Lu	Human HOS	Human TE671
MLV-A	++	++	++	+	++
MLV-E	++	ND	ND	ND	ND
MLV-X	ND	++	++	+	++
GALV	++	ND	−	−	+
SSAV	ND	++	ND	−	−
FeLV-B	ND	++	ND	−	+
RD114	++	++	−	−	−
BaEV	ND	ND	−	−	ND

[a] After viral production. ++, >95% inactivation following 1-h incubation; +, >80% inactivation following 1-h incubation; −, ≤80% inactivation following 1-h incubation; ND, not determined.

serum. The virus produced from NIH 3T3 cells was rapidly inactivated; 5 min of exposure to human serum was sufficient to decrease the viral titer more than 3 log units. The virus produced from Mv-1-Lu cells was also inactivated, though less rapidly; after a 1-h exposure to human serum, the viral titer was decreased by 2 log units. A similar effect of producer cell on viral sensitivity to human serum was observed when LacZ(RD114) produced from either NIH 3T3 or Mv-1-Lu cells was used. Figure 1B shows that the titer of the NIH 3T3 LacZ(RD114) was decreased by 2 log units within 5 min, whereas the Mv-1-Lu LacZ(RD114) was resistant to a 1-h exposure to human serum. The activity in human serum responsible for viral inactivation was heat labile (Fig. 1). The results presented in Fig. 1 demonstrated that two factors could influence the sensitivity of retroviruses to inactivation by human serum. First, the cell from which the virus was produced affected sensitivity, with viruses produced by Mv-1-Lu cells being more resistant to human serum than those produced by NIH 3T3 cells. Second, RD114 produced from Mv-1-Lu cells was considerably more resistant than MLV-A produced by the same cells.

We therefore examined further combinations of producer cells and retroviruses. A 1-h exposure time was selected for experiments, which allowed slower inactivation effects to be monitored. These will be of significance in controlling the stability of virus, and thereby viral infectivity in vivo, as prolonged exposure of cells to retroviruses is necessary for efficient infection. Figure 2A shows that the retroviruses produced from NIH 3T3 cells were all sensitive to inactivation by human serum. These included three helper-free recombinant viruses made from the packaging cell lines GP+E86 (14), GP+E AM12 (13), and PG13 (16). Similarly, viruses produced from the dog cell line Cf2ThS+L− were all sensitive to inactivation by human serum (Fig. 2B). However, while two MLVs produced by Mv-1-Lu cells were sensitive to human serum, RD114, BaEV, and GALV were largely resistant (Fig. 2C). A similar pattern of sensitivity was seen when viruses produced by two human cell lines, HOS and TE671, were exposed to human serum. MLV-A or MLV-X produced by either cell was inactivated, whereas RD114 and BaEV were largely resistant. GALV and FeLV-B were partially resistant, and SSAV, which is closely related to GALV (6), was resistant (Fig. 2D and E). In all experiments except those using MLV-A, GALV, and FeLV-B produced from HOS cells, a heat-labile component of human serum was responsible for viral inactivation (Fig. 2). Therefore, as summarized in Table 1, viruses produced from NIH 3T3 or Cf2ThS+L− were sensitive to human serum, whereas those produced from Mv-1-Lu, HOS, or TE671 cells were at least partially resistant, with the exception of MLV-A and MLV-X. Non-virion-associated factors in virus harvests, which could cross-enhance or cross-inhibit virus inactivation, were not detected in experiments in which an admixture of two viruses was treated with human serum and then plated on appropriate cells to distinguish between the two viruses (data not shown).

TABLE 2. Inactivation of recombinant viruses produced from mink Mv-1-Lu cells

Virus	Relative titer (%)		
	NHS-1	NHS-2	NHS-3
MLV-A (1502)	2.7	5.5	10
RD114	120	116	75
MLV *gag/pol* plus:			
MLV-A (4070) *env*	21	21	18
RD114 *env*	104	94	71
MLV-E *env*	32	23	26

To determine which RD114 viral gene product(s) conferred resistance to human serum, recombinant virions were produced from Mv-1-Lu cells by expressing MLV *gag* and *pol* genes in combination with either the MLV or RD114 *env* gene. Table 2 shows that virions with RD114 envelope were resistant to treatment with three fresh human sera for 1 h, whereas those with either ecotropic or amphotropic MLV envelopes were more sensitive. This result demonstrates that envelope sequences can control sensitivity, in agreement with the assignment of p15E as the viral protein which triggers complement (3).

A correlation was observed between the ability of human serum to inactivate retroviruses and its ability to cause lysis of the respective cell lines. Figure 3 shows that NIH 3T3 and Cf2ThS+L− cells were highly sensitive to lysis by human serum. Mv-1-Lu, HOS, and TE671 cells were resistant to lysis. Viral infection of the cell lines did not affect their sensitivity; in particular, infection of Mv-1-Lu or HOS cells with MLV-A or MLV-X did not render them sensitive to lysis by human serum (Fig. 3). These data suggest that a cellular factor(s), which can protect the uninfected cells from lysis by human serum, may be incorporated into virions produced from mink and human cells. Clearly some viral effect, which does not affect the lysis of infected producer cells, also controls the differential sensitivity of viruses produced from a given cell.

The data in Fig. 1 to 3 were obtained by using several batches of serum from two individuals. Our goal was then to identify virus-producer cell combinations which were universally resistant to inactivation by human serum. Figure 4 presents the results of viral exposure to a panel of eight normal human sera (NHS1 to NHS-8) from healthy volunteers. MLV-A and MLV-X produced from Mv-1-Lu or HOS cells were sensitive to most sera, while RD114 produced from these cells was universally resistant. Results obtained with TE671 cells showed a much more variable pattern of inactivation. No correlation was observed between the blood group of the individual from whom serum was obtained and the ability of the serum to inactivate TE671-derived virus (data not shown). These data suggest that Mv-1-Lu and HOS cells are the most appropriate for the production of virus resistant to the majority of human sera. The discrepancy between the results obtained with HOS and TE671 cells shows that resistance to human serum not only may be species dependent but also may vary between particular cell lines.

To investigate the mechanism of inactivation of sensitive

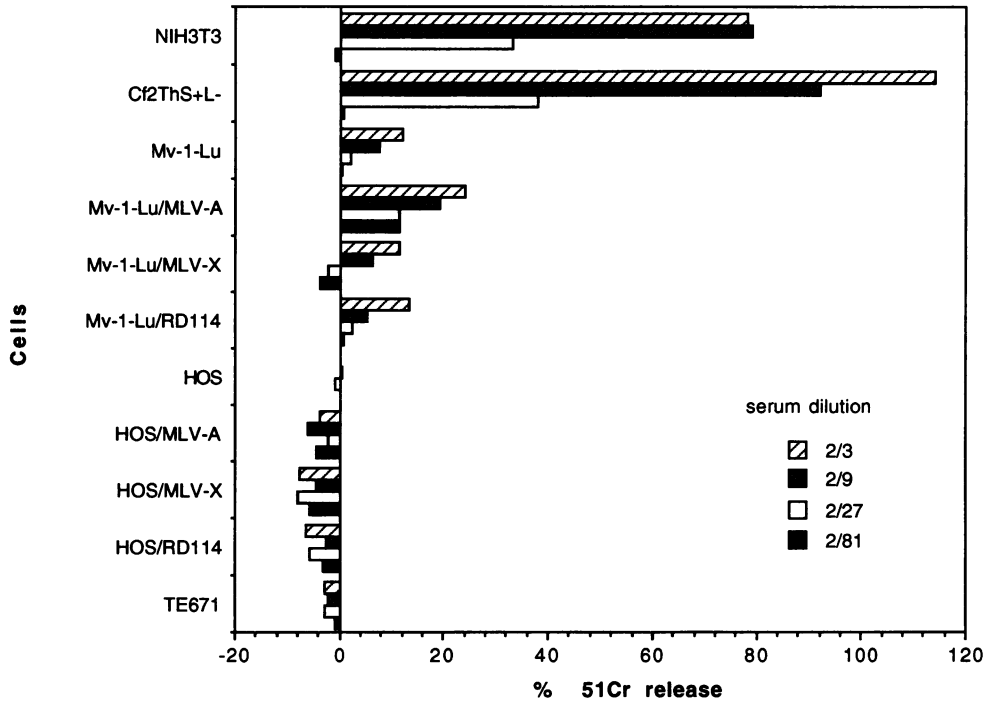

FIG. 3. Cytotoxicity of fresh human serum on cell lines. Cell lines containing MFGnlslacZ provirus, either uninfected or infected with replication-competent viruses (MLV-A, MLV-X, and RD114), were labeled with ^{51}Cr and incubated with fresh human serum (NHS-2). Specific ^{51}Cr release from cells treated with serum dilutions is shown. No significant release by heat-inactivated serum was observed (data not shown).

retroviruses by human serum, sera deficient in complement components were used. Table 3 shows that depletion of complement from normal human serum, by addition of either cobra venom factor or complement component C1, resulted in the loss of its ability to inactivate MLV-A produced from NIH 3T3 cells or Mv-1-Lu cells and RD114 produced from 3T3 cells. Thus, as previously reported (30), the viral inactivation of MLV-A and RD114 by human sera that we observed was due to complement. C1 addition specifically depletes the classical pathway of complement activation. Our data are therefore in agreement with the previous report of involvement of the classical pathway in retroviral inactivation by human serum (5). Complement was present in excess of virions in our experiments, as no depletion of complement components from human serum, following incubations with any of the retroviruses, was observed (data not shown).

The use of sera from individuals deficient in particular complement components allowed us to investigate the mechanism further. C2-deficient serum, which is unable to trigger the classical pathway of complement, also failed to inactivate MLV-A or RD114 (Table 3). However, sera deficient in two of the complement components common to both the classical and alternative pathways C7 and C9 were still effective in viral inactivation (Table 3). C7 and C9 are involved in the final steps of complement-mediated lysis and are components of the membrane attack complex. These data thus demonstrated that a step prior to C7 deposition was sufficient for inhibition of retroviral infectivity. We therefore examined the effects of human sera on retroviral particle lysis by measuring RT release from purified virions. As previously reported (30), normal human serum was able to cause RT release from virions (Table 4). With two normal sera, some correlation was observed

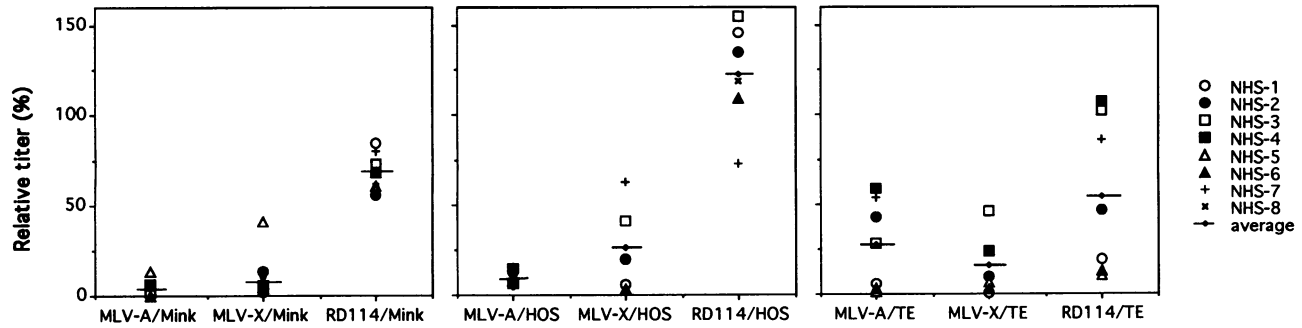

FIG. 4. Virus sensitivity to a panel of human sera. Viruses (MLV-A, MLV-X, and RD114) from Mv-1-Lu (Mink), HOS, and TE671 (TE) cells were treated with fresh normal human sera (NHS-1 to NHS-8) at 37°C for 1 h and then plated on TE671 cells. Relative titers, versus FCS treatment, of surviving *lacZ* pseudotypes are shown together with the mean values of relative titers.

TABLE 3. Inactivation of virus infectivity by human sera deficient in complement components

Serum	Mean surviving virus (%) ± SE (n = 2)		
	3T3/MLV-A	3T3/RD114	Mink/MLV-A
CVF[a] treated NHS-7	130 ± 16	91 ± 1	112 ± 3
NHS-7	0.97 ± 0.50	0.58 ± 0.08	28 ± 16
C1-treated NHS-7	150 ± 13	107 ± 13	96 ± 16
NHS-7 (1/3 diluted)	11.7 ± 2.6	14.1 ± 0.8	37 ± 9
C2D	72[b]	25 ± 8	147 ± 16
C7D-1	<1	0.15	2.5
C7D-2	5.8	1.6 ± 0	7.8 ± 1.4
C9D	<1	<0.2	9.1 ± 3.9

[a] CVF, cobra venom factor.
[b] A single experiment was done.

between the extent of RT release and the inhibition of infectivity, in that MLV-A produced from Mv-1-Lu cells was more sensitive to lysis than RD114, and virus produced from NIH 3T3 cells was more sensitive than that produced from Mv-1-Lu cells (Table 4). However, the C7-deficient serum which inhibited viral infectivity failed to induce virion lysis (Table 4). These data demonstrate that a step in activation of complement by the classical pathway, prior to the final stage of virion lysis, is sufficient for inhibition of retroviral infection by human serum.

DISCUSSION

This report demonstrates that both viral and cellular components control the sensitivity of type C retroviruses to inactivation by human serum. A correlation was observed between the resistance of producer cells to lysis by human serum and their ability to produce resistant virus. Resistance of cells to lysis by complement from the same species is due to the presence in cell membranes of complement control proteins (reviewed in reference 17). The widely distributed membrane cofactor protein (CD46) and decay-accelerating factor limit the activation of complement, while CD59 inhibits membrane attack complex assembly. It is possible that the incorporation of such protective cellular components into virions partly controls the producer cell effect, as type C retroviruses are known to incorporate cellular membrane proteins into the virion membrane (1, 4, 12, 21). However, we have found that expression of CD46, decay-accelerating factor, or CD59 in NIH 3T3 cells can protect the cells from lysis by human serum but does not affect the complement sensitivity of viruses produced from the cells (data not shown). A combination of complement control proteins or further, as yet unidentified molecules may be required.

As retroviral virions contain host membrane proteins, it is also possible that cell surface molecules play a role in complement activation by viruses produced from NIH 3T3, Cf2ThS+L−, and, with certain sera, TE671 cells. When the destruction of virions by complement is said to be antibody independent, this normally implies independent of antibodies to virion proteins. However, human sera are likely to contain naturally occurring heterophile antibodies to carbohydrate antigens on producer cells. Such antibodies should be effective in virion inactivation, whereas selection has ensured that antibodies to viral envelope proteins do not usually fix complement. This preferential neutralization by anticell antibodies has been observed in monkeys immunized with human cell lines, which can then inactivate simian immunodeficiency virus grown in human but no monkey cell lines (25). It has also been shown that natural antibodies against murine cell determinants mediate the inactivation by human complement of the nonretrovirus lymphocytic choriomeningitis virus only when it is produced from mouse cells (29). A further possibility is that differential viral glycosylation, controlled by the producer cell, determines the viral sensitivity to complement. Such effects have been observed for Sindbis virus (8), and retroviral envelope proteins are known to show changed glycosylation in different producer cells (10).

TABLE 4. Virus lysis by human sera deficient in complement components

Serum	RT release (% Triton X-100)			
	3T3/MLV-A	3T3/RD114	Mink/MLV-A	Mink/RD114
NHS-7	39 ± 4	13 ± 5	21 ± 5	3 ± 1
NHS-3	150 ± 1	61 ± 15	63 ± 2	16 ± 2
C7D-2	−0.7 ± 0.3	7 ± 8	−6 ± 1	−7 ± 2

The differential sensitivity of several retroviruses produced from the same cell line allows further analysis of the control of viral triggering of human complement. Here we demonstrate that the viral *env* gene products determine virion sensitivity, in agreement with the assignment of p15E as the viral protein which triggers complement (3). Further analysis of the complement sensitivity conferred by chimeric envelope proteins will allow us to determine the precise location of sequences responsible for triggering complement. RD114 and BaEV, both of which show envelope sequence homology to type D retroviruses (11, 19) and share the type D receptor (23, 24), are resistant to inactivation by human serum. It is of interest that the closely related viruses SSAV and GALV show differential sensitivity. SSAV is believed to have arisen from zoonotic infection of a woolly monkey with GALV (6). While the two viruses show a high degree of sequence homology and share the same cell surface receptor (28), subtle differences in their receptor sequence requirements have been shown (26). Thus, minor sequence differences can also control viral sensitivity. The extent to which complement control proteins are effective in protection is known to relate to the rate of complement activation. This finding suggests that the rate of complement activation by the sensitive MLVs, which can be inactivated even when produced from human or mink cells, may be more rapid.

The mechanism of retroviral inactivation by human serum remains unclear. Although RT release has often been observed (30, 31) and characteristic complement lesions in virions have been identified by electron microscopy (3), we have found that RT release is not necessary for viral inactivation. From the observation that incubation with C7-deficient sera inhibits infection, we can conclude that deposition of a complement component(s) prior to C7 inhibits type C retrovirus infectivity. In contrast, in the case of human immunodeficiency virus, complement fixation can allow viral infection via complement receptors (20).

Our results demonstrate that effects of the retroviral producer cell line must be considered when viral inactivation by serum is measured. SSAV and RD114 produced from human A204 or Raji cells have previously been shown to be sensitive to human complement (22, 31). This is not inconsistent with our findings, as a panel of human sera consistently failed to inactivate RD114 produced from HOS cells while having a variable effect on RD114 produced from TE671 cells. As cell line-specific effects can occur, a reevaluation of viral inactivation by sera of susceptible hosts will be of interest. Much

previous work in this area has not considered the presence of producer cell-specific effects. It is possible that efficient endogenous retroviral production, or horizontal transmission, occurs only when virus is produced from cells which confer resistance to host serum.

All mammalian retroviral packaging cell lines that have been constructed have been based on murine NIH 3T3 cells. They therefore produce recombinant viruses which are sensitive to inactivation by human serum (this report). It is possible to supply the *gag/pol* and *env* genes on separate plasmids in packaging cells, which considerably reduces the risk of recombination with vector sequences and prevents the generation of replication-competent virus. As we have identified cell lines which consistently produce retroviruses resistant to human serum, we can now make packaging cell lines capable of producing replication-defective retroviruses. We have demonstrated that a wide variety of type C retroviruses, including RD114, BaEV, and SSAV, will package existing MLV-based vectors (references 26 and 28 and this report) and that diverse retroviral envelope proteins can also be expressed in combination with existing MLV *gag/pol* constructs. This will allow the rapid generation of a variety of recombinant, helper-free retroviruses, resistant to human serum, suitable for clinical, in vivo gene delivery.

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