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Detection of Receptor-Specific Murine Leukemia Virus Binding to Cells by Immunofluorescence Analysis

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Four classes of murine leukemia virus (MuLV) which display distinct cellular tropisms and bind to different retrovirus receptors to initiate virus infection have been described. In the present study, we describe a rapid, sensitive immunofluorescence assay useful for characterizing the initial binding of MuLV to cells. By using the rat monoclonal antibody 83A25 (L. H. Evans, R. P. Morrison, F. G. Malik, J. Portis, and W. J. Britt, J. Virol. 64:6176–6183, 1990), which recognizes an epitope of the envelope gp70 molecule common to the different classes of MuLV, it is possible to analyze the binding of ecotropic, amphotropic, or xenotropic MuLV by using only a single combination of primary and secondary antibodies. The MuLV binding detected by this assay is envelope receptor specific and matches the susceptibility to infection determined for cells from a variety of species. The binding of amphotropic MuLV to NIH 3T3 cells was shown to be rapid, saturable, and temperature dependent. Chinese hamster ovary (CHO-K1) cells normally lack the ability to bind ecotropic virus and are not infectible by ecotropic vectors. Expression of the cloned ecotropic retrovirus receptor gene (*Rec*) in CHO-K1 cells confers high levels of ecotropic virus-specific binding and confers susceptibility to infection. Characterization of MuLV binding to primary cells may provide insight into the infectibility of cells by retroviruses and aid in the selection of appropriate vectors for gene transfer experiments.

Gene transfer with modified retroviruses as vectors has been an important tool for molecular and cellular biologists for nearly a decade (4). To date, the Moloney murine leukemia virus (Moloney MuLV) has proven to be the most useful as a gene transfer vector for mammalian cells because of its relative simplicity and the ease with which it can be genetically modified. As the technology has developed, more complex vector constructions and sophisticated packaging systems have broadened the application of retrovirus-mediated gene transfer to a wide range of biological systems (12, 28). Most recently, this technology has found clinical application for transferring genes into primary human cells to mark cells (37), correct specific genetic defects (8), and deliver specific lymphokines in vivo (36).

Despite these advances, some aspects of the retroviral life cycle are still only poorly understood. As a result, the utility of retroviral vectors for gene transfer into new cell systems must often be determined by trial and error. Before we can eliminate this empirical aspect of the technology, a better understanding of the process of retroviral penetration of host cells, integration of proviral DNA, and subsequent gene expression is necessary. One area of particular interest has been the identification of specific retroviral receptors on the cell surface which are necessary for MuLV attachment and subsequent infection (2, 17, 21). Previous efforts to characterize the initial virus-cell interaction have required the preparation of either radiolabeled purified virus (3) or directly fluoresceinated purified virus preparations (26, 27). Other studies have characterized the binding of purified iodinated envelope glycoprotein to cells and cell membranes (7, 10, 11, 15, 19, 20).

We report here a rapid, sensitive assay that is useful for characterizing the binding of a variety of different murine retroviruses to potential target cells. The method consists of an indirect immunofluorescence assay which couples a broad-specificity anti-envelope (gp70) monoclonal antibody (MAb) with fluorescence flow cytometry. The virus preparations required for this assay are identical to those used for standard retroviral gene transfer experiments, and a single combination of primary and secondary antibodies can be used to detect the binding of MuLV with ecotropic, amphotropic, or xenotropic envelope specificities. Our results suggest that this assay may be useful for quickly determining the potential for infection of cells by the different classes of MuLV.

MATERIALS AND METHODS

Cell lines. Cell lines were maintained in an incubator at 37°C under 5% CO_2. NIH 3T3 cells (ATCC CRL1658) (18), mink lung fibroblasts (ATCC CCL64) (16), and the human epithelioid carcinoma line HeLa (ATCC CCL2) (38) were grown in D10 (Dulbecco's modified Eagle medium) (containing 4.5 g of glucose per liter, supplemented with 2 mM L-glutamine and 10% fetal bovine serum [FBS]). Chinese hamster ovary cells (CHO-K1; ATCC CCL61) (35) were grown in alpha minimal essential medium–2 mM L-glutamine–10% FBS. The human myelogenous leukemia line K-562 (ATCC CCL243) (23) was maintained in RPMI–2 mM L-glutamine–10% FBS. The rat hybridoma cell line 83A25 (14) was maintained in RPMI–2 mM L-glutamine–1% FBS–1% Nutridoma-Hu (Boehringer-Mannheim, Indianapolis, Ind.). Human primary lymphocytes were grown in AIM-V (GIBCO BRL, Gaithersburg, Md.) supplemented with 5% FBS and 1,000 U of recombinant human interleukin-2 per ml.

Viruses and virus producer cells. All virus producer cells were grown in D10. Ecotropically packaged retroviral vectors were generated from the F5B cell line, which consists of a recombinant provirus containing the neomycin resistance gene (Neo^r) (N2) (5) in the psi-2 packaging cell line (25).

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Amphotropically packaged vectors, also containing the Neo^r gene (LNL6 [6] and G1N [29]), were generated with the PA317 packaging cell line (30). Supernatants containing the packaged vectors were prepared by harvesting medium from confluent monolayers of virus producer cell lines fed 24 h previously. Supernatants were cleared by centrifugation (2,000 × *g* for 10 min), portioned, and frozen at −70°C until use. The concentration of infectious particles in the virus preparations was determined by standard titer determination methods based on selection for the neomycin resistance gene (13). Amphotropic virus supernatants used in this study contained 1×10^6 to 5×10^6 CFU/ml; ecotropic virus supernatants had an average titer of 5×10^5 CFU/ml.

Transfection and Selection of CHO/pJET cell lines. CHO cells (2×10^5) were seeded in a 10-cm dish on the day prior to use. The cells were cotransfected with 29 μg of the murine ecotropic receptor plasmid pJET (2) plus 1 μg of the hygromycin resistance marker pY3 (9) by the calcium phosphate precipitation method (41). Approximately 24 h later, the cells were rinsed free of DNA precipitate and cultured for an additional 24 h, at which time 500 μg of hygromycin B (Boehringer-Mannheim) per ml was added to the medium. Selection was continued for 12 to 14 days until hygromycin-resistant colonies were visible. Colonies were isolated with glass cloning cylinders, expanded in culture, and tested for infectibility by ecotropic MuLV.

Antibodies. For MAb preparation, 48-h cultures of hybridoma supernatant were collected. Antibody supernatant was concentrated approximately 10-fold by using Centricon 30 (Amicon, Beverly, Mass.) filter units as specified by the manufacturer. A phycoerythrin-conjugated goat $F(ab')_2$ anti-rat immunoglobulin was commercially obtained (Tago, Burlingame, Calif.). Appropriate levels of primary and secondary antibody were determined by measuring the titer on antigen-positive PA317 viral packaging cells. Generally, 60 μl of the concentrated MAb supernatant and 1 μl (0.5 μg) of PE-conjugated $F(ab')_2$ were used to stain 10^6 cells. Purified rat immunoglobulin G2a was obtained from Zymed, San Francisco, Calif., as an isotypic control for MAb 83A25.

Virus-binding assay. Cells to be tested for virus binding were harvested by brief trypsinization, rinsed with serum-containing medium, and portioned to assay tubes (10^6 cells per sample unless otherwise indicated). The cells were pelleted in 4-ml polypropylene (Falcon no. 2063) tubes (250 × *g* for 5 min) and resuspended in 1 ml of medium containing the appropriate dilution of virus supernatant plus 8 μg of polybrene per ml. They were incubated at 37°C for 40 min (unless otherwise indicated) with occasional agitation to maintain a cell suspension. Following incubation with virus, cells were washed twice with 2 ml of ice-cold phosphate-buffered saline (PBS)–5% FBS. The first wash was added directly to the binding-reaction mixture. The cells were resuspended in 60 μl of MAb and incubated on ice for 40 min. They were again washed twice with 2 ml of ice-cold buffer and resuspended in 100 μl of PBS–5% FBS containing 1 μl (0.5 μg) of phycoerythrin-conjugated $F(ab')_2$. They were incubated on ice in the dark for 40 min, washed once with 2 ml of cold buffer, and resuspended to a final concentration of approximately 10^6 cells per ml of PBS–1% paraformaldehyde. Following virus binding and antibody staining, cell samples were analyzed for fluorescence intensity on an Epics C flow cytometer (Coulter). Cells fixed in PBS–1% paraformaldehyde prior to virus binding were also tested. Although specific virus binding was still observed, the effect of this treatment was variable among the different cell lines tested and the amount of virus binding was reduced. In all of the studies reported here, fresh, unfixed cells were used.

RESULTS

In preliminary experiments, we determined that the rat monoclonal antibody 83A25 (14), which has a broad specificity for MuLV gp70, could be used in an indirect immunofluorescence assay to detect the initial binding of murine retroviruses to the surface of various types of mammalian cells. To determine whether the virus binding detected by this method represents a specific viral envelope-cell surface receptor interaction, we compared the binding specificity of ecotropic and amphotropic viruses on mouse NIH 3T3, human HeLa, and hamster CHO-K1 cells (Fig. 1). Cells harvested and incubated in medium without virus prior to antibody staining represent antibody controls. NIH 3T3 cells, which are readily infectible by both ecotropic and amphotropic MuLV, show a 10-fold increase in fluorescence intensity resulting from the binding of either virus (Fig. 1). Human cells are infectible by amphotropic MuLV only. The virus-binding pattern observed for HeLa cells agrees with this observation, since only amphotropic virus binding is detectable. CHO-K1 cells, which are not infectible by either ecotropic or amphotropic MuLV, show no binding of either virus. Three additional cell lines were evaluated for both virus binding and infectibility (Table 1). The level of binding and degree of infectibility among these cell lines was variable, but in all cases, if binding was observed, infection by appropriately packaged vector was also detected.

Because these studies were carried out with normally adherent fibroblast cell lines which were removed from their culture substrate by mild trypsinization, we investigated the possibility that the virus-binding profile is altered or diminished by this treatment. Several lines of evidence indicate that mild trypsinization does not alter the virus-binding properties or infectibility of cells (data not shown). First, we observed no qualitative or quantitative difference in virus binding to NIH 3T3 cells harvested by trypsinization or mechanically dislodged from the culture dish. Second, virus binding to the nonadherent cell line K-562, which is comparable to that shown for HeLa cells in Fig. 1, was unaltered by trypsin treatment of cells. Lastly, titer experiments performed with adherent NIH 3T3 cells and with NIH 3T3 cells trypsinized and maintained in suspension during virus infection yielded comparable virus titers.

To further assess the nature of the virus-cell interaction detected by this method, we investigated several of the parameters that are used to characterize a receptor-ligand interaction, including the saturability, time course, and temperature dependence of binding. For these studies we focused on the binding of amphotropic MuLV to NIH 3T3 cells. An increasing virus concentration resulted in an increase in cell-bound fluorescence (Fig. 2A). Concentration-dependent virus binding to NIH 3T3 cells at 37°C was investigated over a 333-fold virus concentration range (Fig. 2B). Virus binding to NIH 3T3 cells yields a linear increase in mean channel fluorescence between 3 and 30 μl of virus (5×10^6 CFU/ml) per ml of cells, reaching a maximum at 100 μl of virus per ml. This binding is saturable, with no increase in fluorescence intensity resulting from an additional 10-fold increase in virus concentration (Fig. 2B).

Several results indicate that the plateau in the mean channel fluorescence (Fig. 2B) represents true saturable binding and is not due to depletion of either virus or antibodies. At virus saturation, increasing the sample cell

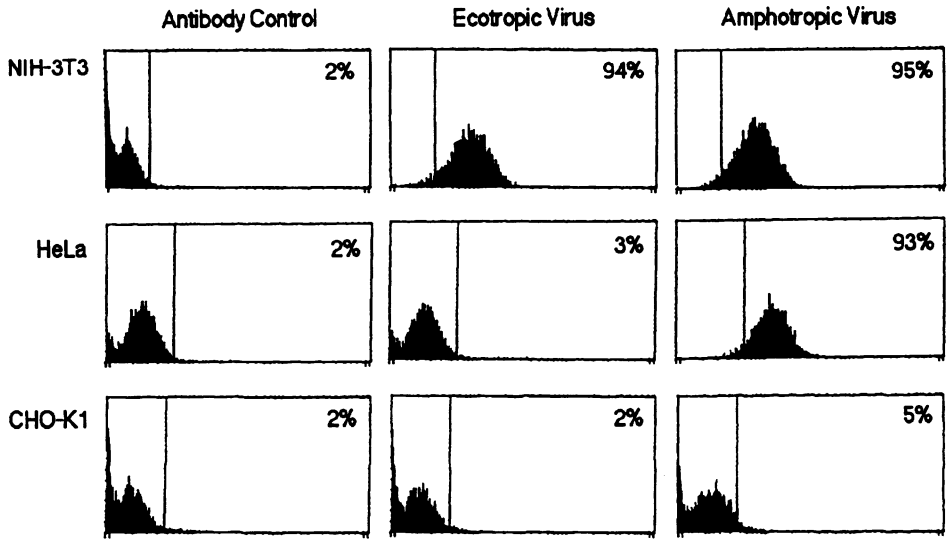

FIG. 1. Specificity of ecotropic and amphotropic MuLV binding to human and rodent cells. The x-axis is log red fluorescence, measuring the amount of phycoerythrin labeling per cell; the y-axis is the number of events detected. All samples shown were analyzed at the same red photomultiplier gain. Control cells were used to establish cellular fluorescence background, and a cursor is set just to the right of the negative cell population. For each histogram, the percentage in the upper right corner indicates the percentage of the population to the right of the cursor.

number fivefold did not significantly decrease the mean fluorescence intensity of the cell population. In addition, increasing the concentrations of both primary and secondary antibodies during staining had no effect on the fluorescence intensity of the samples. Additional control experiments to determine the assay specificity were performed. Incubating cells with culture medium collected from NIH 3T3 cells had no effect on the fluorescence of the cell population compared with incubating cells in fresh culture medium alone. In addition, substituting an isotype-matched antibody for the anti-gp70 MAb abolished the increase in fluorescence observed for NIH 3T3 cells incubated with 1 ml of virus supernatant (data not shown).

The rate of virus binding to 3T3 cells was determined at 37 and 4°C (Fig. 3). For these experiments, virus (1.5×10^6 CFU/ml) was equilibrated to 37 or 4°C prior to mixing with cells. Virus binding in the 37°C samples was terminated at the appropriate time by the addition of 10 volumes of ice-cold wash buffer directly to the binding-reaction mixture. Virus binding at 37°C is very rapid. At virus saturation (as determined by Fig. 2B), half-maximal binding occurs at approximately 2 min and binding reaches equilibrium at 10 min. The amount of virus antigen detectable on the cell surface shows little change between 10 and 60 min at 37°C. The detection of virus binding is highly temperature dependent. The mean channel fluorescence of cells incubated with virus for 60 min at 4°C increases only 1.6-fold relative to the 4°C control cells with no virus. In contrast, cells incubated for 20 min at 37°C with the same concentration of virus show nearly a 7-fold increase in fluorescence (Fig. 3). Cells that are incubated with virus first at 4°C and then shifted to 37°C while still mixed with the virus show binding equivalent to that of samples incubated at 37°C only (data not shown).

The gene for the murine ecotropic retroviral receptor (*rec*) has been cloned (2). Thus we could directly test the effect of expressing a receptor gene with a known envelope specificity in cells which show no virus binding. CHO-K1 is an appropriate cell line for this experiment, since it is normally

TABLE 1. Comparison of virus binding and infectibility

Cell Line	Species, tissue type	MuLV binding[a]		Cell infectibility (%)[b]	
		Ecotropic	Amphotropic	Ecotropic	Amphotropic
NIH 3T3	Mouse, fibroblast	11.8	10.8	>90	50
HeLa	Human, cervical carcinoma	1.0	8.2	0	3
K-562	Human, leukemia	1.0	5.3	0	2–5
Lymphocytes	Human, primary	1.0	2.3	0	1–10
MV 1 Lu	Mink, lung fibroblast	1.0	1.6	0	25
CHO-K1	Chinese hamster, ovary	1.0	1.0	0	0

[a] Virus-binding assays were performed as described in Materials and Methods, with 10^6 cells per sample and 1 ml of undiluted viral supernatant. MuLV binding values are the fold increase in fluorescence intensity (FI) calculated as the ratio of sample FI to control FI. FI values correspond to the x-axis log scale and were derived from linear mean channel values by using a 256-channel to three-decade log conversion table. Each value is the average from three or more experiments.

[b] Values for cell infectibility are the percentage of cells transduced (average value from three or more experiments per cell line) following a 4-h infection at a multiplicity of infection of 10. Several methods were used to establish cell infectibility. For adherent cell lines (NIH 3T3, HeLa, MV 1 Lu, and CHO-K1), cells were fixed and stained for β-galactosidase activity 2 days after exposure to viral vectors containing the *lacZ* gene. K-562 cells were also transduced with *lacZ*-containing vectors and assayed by using fluorescein-di-β-galactopyranoside as a substrate in a flow cytometric assay (31). Alternatively, K-562 cells were transduced with vectors containing the Neo^r gene and colony assays were performed by selecting cells in G418 while they were growing immobilized in methylcellulose. The infectibility of human lymphocytes was estimated by using a semiquantitative polymerase chain reaction method to detect vector sequences.

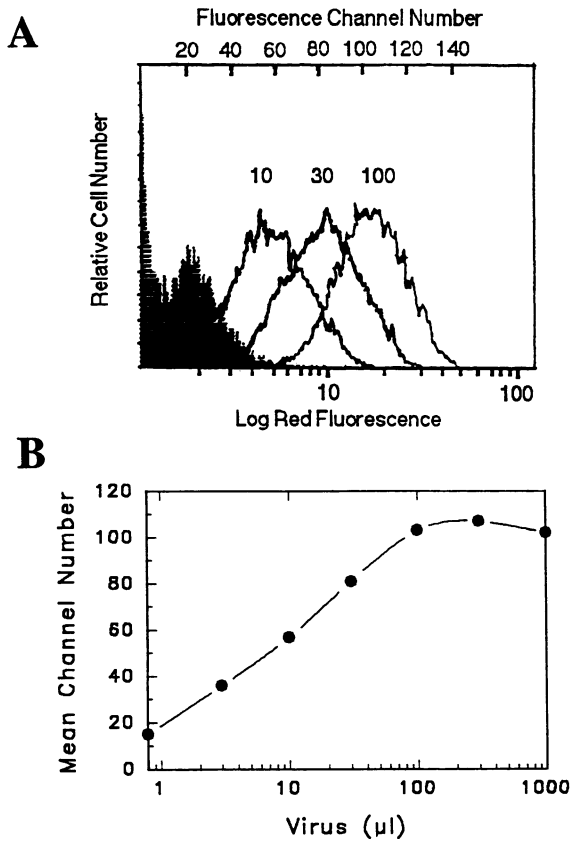

FIG. 2. Concentration-dependent binding of amphotropic MuLV to NIH 3T3 cells. (A) The fluorescence histograms of cells incubated with 10, 30, and 100 μl of virus per ml (shown just above the sample peaks) are overlaid with a control sample (darkened-peak) histogram to depict the shift in population fluorescence intensity with increasing virus concentration. Log red fluorescence and fluorescence channel number are shown on the bottom and top *x*-axis, respectively, for comparison. (B) Virus binding was assayed over a 333-fold virus concentration range from 3 μl of virus per ml to 1 ml of undiluted virus. The mean fluorescence intensity for each sample is plotted as mean channel number (*y*-axis) versus the volume (microliters) of input virus per milliliter (*x*-axis).

uninfectible by either ecotropic or amphotropic MuLV and is also negative for binding of both viruses (Fig. 1). CHO-K1 cells were cotransfected with the *rec* expression plasmid pJET, and a hygromycin resistance plasmid and clones were generated as described in Materials and Methods. Cell lines expressing the ecotropic receptor were identified by their infectibility with the ecotropic vector psi-2 BAG (34). We identified several cell lines which were infectible at a frequency of about 50%, comparable to the infection frequency of NIH 3T3 cells with this vector. These cell lines were uninfectible by amphotropic vectors of equivalent titer. Two such CHO/pJET transfected cell lines were assayed for their ability to bind ecotropic or amphotropic MuLV (Fig. 4). Both cell lines, designated CHO/pJET#2 and CHO/pJET#11, show a marked increase in mean fluorescence intensity following incubation with ecotropic virus, with no detectable change in intensity for the parent CHO cells. In contrast, incubation with amphotropic MuLV had no effect on the fluorescence profile of either CHO/pJET cell line or control CHO cells (Fig. 4).

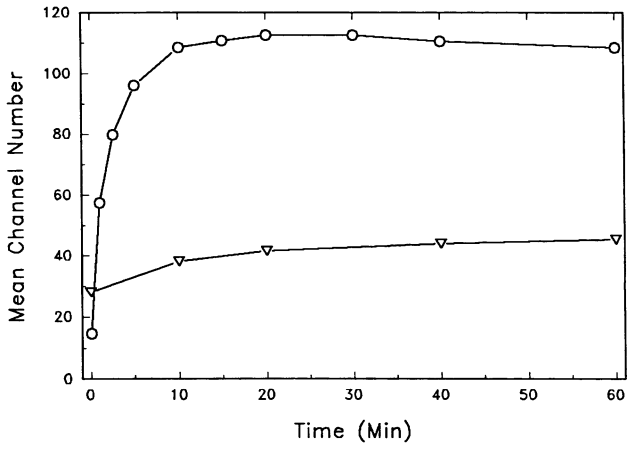

FIG. 3. Time course and temperature dependence of virus binding to NIH 3T3 cells at 37°C (○) or 4°C (▽). Virus binding in the 37°C samples was terminated at the appropriate time by the addition of 10 volumes of ice-cold wash buffer.

DISCUSSION

The rat MAb 83A25 recognizes an epitope within the envelope gp70 molecule common to all classes of MuLV and is useful for the direct quantitative assay of cells productively infected by ecotropic, xenotropic, polytropic, or amphotropic MuLV (14). In this study we have shown that MAb 83A25 is also a useful reagent for detecting the binding of MuLV to host cells. By combining this broadly reactive yet highly specific anti-gp70 MAb with the sensitivity of fluorescence flow cytometry, we can readily detect envelope-specific virus binding of recombinant ecotropic and amphotropic MuLV to appropriate target cells. In preliminary experiments with wild-type xenotropic virus from a productively infected mink cell line, we have also detected xenotropic virus binding to mink and human cells.

The binding of ecotropic and amphotropic MuLV to human and rodent cells matches the pattern predicted by the host range of MuLV infectibility. That is, murine fibroblasts (NIH 3T3) bind both ecotropic and amphotropic virus, human HeLa cells bind only amphotropic virus, and uninfectible fibroblast lines such as CHO-K1 bind neither (Fig. 1). These results suggest that the pattern of virus binding observed by using this assay may be useful for predicting the viral tropism of an uncharacterized cell line. The correlation observed between virus binding and infectibility has been observed for a variety of cells and cell lines, including the human suspension cell line K-562, primary human lymphocytes, and human fibroblasts (HeLa), as well as mink, mouse, and hamster fibroblast lines (Table 1). It should be noted, however, that binding of virus to its receptor is only the first in a series of necessary events leading to infection. Therefore there may be cases in which cells displaying high levels of specific virus binding are resistant to infection as a result of a block at some subsequent step in the retrovirus life cycle. Indeed, the data in Table 1 show that high levels of virus binding are not sufficient to ensure a high frequency of infection for that cell type. Human cells such as K-562 and HeLa exhibit virus-binding levels of 50 to 80% that of NIH 3T3 cells. However, these human cells infect at a frequency 10% or less of NIH 3T3 cells, as shown by our data (Table 1) as well as by Lynch and Miller with HeLa cells (24). Other cells, such as the mink fibroblast line MV 1 Lu, bind only modest amounts of virus compared with the human cell lines

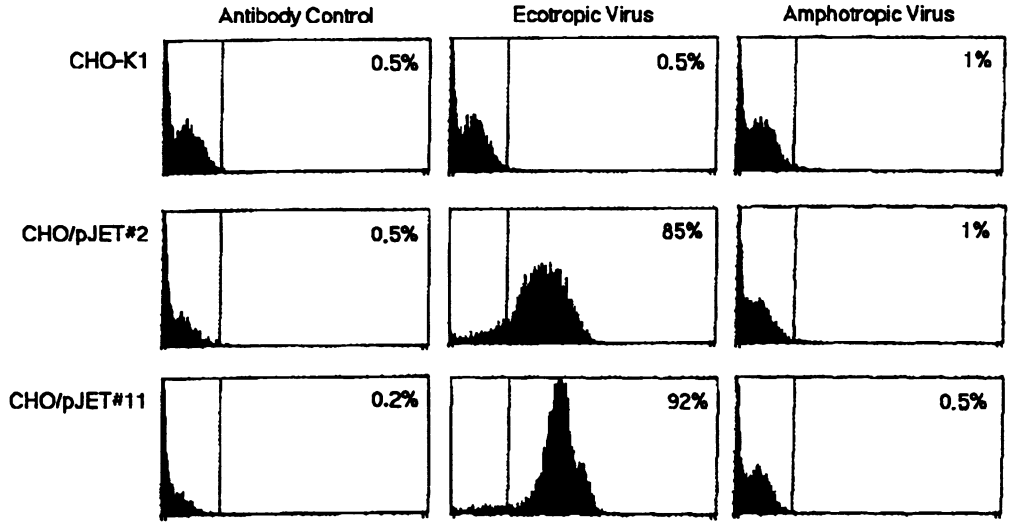

FIG. 4. Virus-binding properties of CHO-K1 cells expressing the murine ecotropic MuLV receptor gene (*rec*). Wild-type CHO-K1 cells or CHO-K1 cells transfected with the *rec* expression plasmid pJET (CHO/pJET#2, CHO/pJET#11) were tested for their capacity to bind MuLV. Control cell samples, incubated in medium without virus, were used to establish background fluorescence at approximately the 1% level. For each histogram, the percentage in the upper right corner indicates the percentage of the population to the right of the cursor. The *x*-axis is log red fluorescence intensity, and the *y*-axis represents the relative cell number for each histogram.

in Table 1, but infect 5 to 10 times more efficiently. Therefore the relative number of virus-binding sites on a cell is not an absolute indicator of its infectibility. However, for all the cell lines we have studied, the presence or absence of virus binding correlates qualitatively with the infectibility of the cells.

Several characteristics of the virus binding presented here suggest that it is receptor specific. The binding is envelope specific (e.g., amphotropic but not ecotropic virus binds to HeLa cells), suggesting that it is mediated by a specific viral envelope-receptor interaction. If adsorption of MuLV to cells were a nonspecific phenomenon, the amino acid sequence differences between ecotropic and amphotropic MuLV envelopes would not be expected to alter the tropism exhibited by these viruses. Furthermore, infectible and noninfectible cells would probably show similar levels of virus binding. Our results (Fig. 1) indicate that ecotropic and amphotropic MuLV bind to potential host cells via highly envelope-specific receptors and do not adsorb nonspecifically to the surface of cells not expressing these receptors. These results are in contrast to the broad, nonspecific binding to infectible and noninfectible cells observed with some avian retroviruses (32, 39, 40).

Another hallmark of receptor-specific binding is that the binding should be saturable with increasing "ligand" concentration. We have characterized the binding of amphotropic MuLV to NIH 3T3 cells and found it to be concentration dependent over approximately 2 logs of virus concentration and saturable (Fig. 2), indicating that only a finite number of specific sites for virus attachment exist. These results are qualitatively similar to previous reports of intact MuLV particle binding assayed by using directly fluoresceinated viruses (26). It is difficult, however, to determine the virus/cell ratio at which saturation was achieved in these previous studies. In the current study we have used recombinant viral vectors of known titer, making it possible to assess the saturation of virus binding in terms of increasing multiplicity of infection. Our results indicate a surprisingly rapid saturation of virus binding to NIH 3T3 cells with respect to the infectious virus/cell ratio (Fig. 2). Maximal binding to 5×10^5 cells is observed with 100 μl of a 5×10^6-CFU/ml viral supernatant, suggesting saturation for the binding of virus or virus antigen at a multiplicity of infection of 1.0 (Fig. 2B). Several aspects of this observation should be considered. For retroviruses in general, the total virus particle concentration in an inoculum far exceeds the infectious-virus concentration. For MuLV the ratio of infectious to total particles is between 0.5 and 1.0% (3). Therefore, at a multiplicity of infection of 1.0, the virus/cell ratio is likely to be 100:1 or greater. In addition, it is likely that each virus binds multivalently to cells, with possibly tens or hundreds of viral gp70 molecules per virion binding independent cellular receptors (22). Another factor contributing to the saturability of virus binding may be free (non-virus-associated) gp70 in the viral supernatant. Free gp70 could arise as a result of loss from virions or direct secretion by virus producer cells and would contribute to the apparent virus binding since it would also be recognized by MAb 83A25. Indeed, binding of purified Moloney MuLV gp70 to NIH 3T3 cells is readily detected by this assay (data not shown).

Amphotropic MuLV binds rapidly at 37°C and is highly temperature dependent (Fig. 3). The plateau in mean channel fluorescence observed between 10 and 60 min indicates no change in the amount of cell-bound virus antigen. These results suggest that the initial cell surface binding of virus or virus envelope occurs much more rapidly than their subsequent clearance from the cell surface and/or that once binding has reached apparent equilibrium, the rate of additional virus binding equals the rate of clearance from the cell surface.

In other experiments we have determined the time dependence of infection for our viral vectors by varying the time of virus exposure in our standard titer procedure. We observed that virus preparations with a titer of 10^6 CFU/ml after 4 h of exposure at 37°C already yielded 10% of this titer after just 5 min of exposure. We would expect the rate of initial virus binding to equal or exceed the time course for infection. Thus, the high rate of virus association in Fig. 3 (maximal

binding in less than 20 min) is consistent with the time course of the biological response (i.e., infection) resulting from this binding. In addition, these data agree with the association kinetics of intact virions as determined by monitoring viral reverse transcriptase activity adsorbed to cells (1).

The kinetics of association for many ligand-receptor interactions are reduced at temperatures below 37°C. We observe a marked decrease in amphotropic MuLV binding at 4°C compared with that at 37°C (Fig. 3). Previous studies with intact MuLV have also found decreased virus binding rates at 4°C (1, 3). Each MuLV particle is thought to bind multivalently to many individual cellular envelope receptors; this receptor-clustering process would be inhibited at 4°C since receptors could not readily migrate within the cell membrane (22, 33). Therefore, at 4°C the virus may be binding only monovalently. It is likely that the extreme reduction in virus binding we observe at 4°C results in part from loss of monovalently or weakly bound virus during antibody binding and subsequent wash steps.

The availability of the cDNA encoding the murine ecotropic retroviral receptor (2) enabled us to test the receptor specificity of the virus-binding assay. The *rec* gene was expressed in CHO-K1 cells, and experiments confirmed that this gene confers efficient infection by ecotropic MuLV on these normally uninfectible cells. This study also shows that expression of *rec* confers envelope-specific binding, in that ecotropic but not amphotropic MuLV will bind to the surface of CHO cells expressing the *rec* gene. These results demonstrate the precise envelope receptor specificity of this assay, as well as the low level of nonspecific virus adsorption to receptor-negative cells. In addition, these data provide further proof of the viral receptor role of the *rec* gene.

In this communication we describe a rapid, sensitive assay that is useful for analyzing the binding of most MuLV to potential host cells. The binding detected by this method is envelope receptor specific and saturable and may be useful for predicting the infectibility of cells by different classes of MuLV. Thus, this assay may be useful for screening primary cells to determine appropriately packaged vectors for retrovirus-mediated gene transfer experiments. Combining the virus-binding assay with fluorescence-activated cell sorting will also enable the identification and isolation of virus-binding subpopulations of cells from complex cell mixtures such as bone marrow. This method provides a useful bioassay for expression of retroviral receptor genes, and the approach we describe should be readily adaptable to other viral systems for which a specific antiviral antibody is available.

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