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Ecotropic Murine Leukemia Virus-Induced Fusion of Murine Cells

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Extensive fusion occurs upon cocultivation of murine fibroblasts producing ecotropic murine leukemia viruses (MuLVs) with a large variety of murine cell lines in the presence of the polyene antibiotic amphotericin B, the active component of the antifungal agent Fungizone. The resulting polykaryocytes contain nuclei from both infected and uninfected cells, as evidenced by autoradiographic labeling experiments in which one or the other parent cell type was separately labeled with [^{3}H]thymidine and fused with an unlabeled parent. This cell fusion specifically requires the presence of an ecotropic MuLV-producing parent and is not observed for cells producing xenotropic, amphotropic, or dualtropic viruses. Mouse cells infected with nonecotropic viruses retain their sensitivity toward fusion, whereas infection with ecotropic viruses abrogates the fusion of these cells upon cocultivation with other ecotropic MuLV-producing cells. Nonmurine cells lacking the ecotropic gp70 receptor are not fused under similar conditions. Fusion is effectively inhibited by monospecific antisera to gp70, but not by antisera to p15(E), and studies with monoclonal antibodies identify distinct amino- and carboxy-terminal gp70 regions which play a role in the fusion reaction. The enhanced fusion which occurs in the presence of amphotericin B provides a rapid and sensitive assay for the expression of ecotropic MuLVs and should facilitate further mechanistic studies of MuLV-induced fusion of murine cells.

A common feature of a variety of enveloped viruses is their ability to induce fusion of cells bearing appropriate receptors. This activity has been observed for myxoviruses, paramyxoviruses, togaviruses, rhabdoviruses, and herpesviruses (40, 41), as well as for retroviruses of murine, bovine, primate, and human origin (7, 8, 20, 29, 38, 42). Previous studies of fusion by murine retroviruses have been performed with several rat cell lines (17, 20, 22, 52, 53) or, more recently, with a cell line derived from tail skin of *Mus dunni* (24, 39); under similar conditions, other murine cells are not fused by these viruses. We now demonstrate that in the presence of the polyene antibiotic amphotericin B, rapid and efficient fusion of a variety of murine cell types occurs upon cocultivation with cells producing ecotropic murine leukemia viruses (MuLVs), and we report preliminary characterization of the viral specificity and mechanism of MuLV-induced fusion of murine cells under these conditions.

MATERIALS AND METHODS

Cell lines and reagents. The cell lines used in this study are listed in Table 1. Pure amphotericin B was provided by E. R. Squibb & Sons, and Fungizone (amphotericin B plus deoxycholate) was obtained from Squibb or GIBCO Laboratories. Monoclonal antibodies and hyperimmune antisera were as previously described (37).

Fusion assays. Fusion was generally induced by cocultivating equal numbers of infected and uninfected cells in Dulbecco minimal essential medium containing heat-inactivated 5% calf serum and 5% fetal calf serum plus appropriate concentrations of either Fungizone or pure amphotericin B. In a typical experiment, 1.0×10^5 infected cells were plated in 35-mm-diameter petri dishes and overlaid the following day with 2.0×10^5 uninfected target cells in medium containing amphotericin B. Similar results were obtained when the two cell lines were plated in reverse order or at the same time and when the cells were grown continuously in the presence of amphotericin B. After cocultivation for periods ranging from 6 to 24 h, fused monolayers were either directly photographed under phase contrast or fixed by treatment with methanol for 5 min, followed by staining with 0.1% cresyl violet in water. Quantitation of fused cells was performed by counting the average number of polykaryocytes present in at least six separate areas of 0.025 cm^2 each, multiplying this number by the average number of nuclei per polykaryocyte, and normalizing for an area of 1 cm^2.

Autoradiography. MuLV-infected cells in the logarithmic phase of growth were labeled for 16 h with [^{3}H]thymidine at 0.1 μCi/ml and then harvested after two washes with Eagle minimal essential medium containing 10^{-5} M cold thymidine. They were then seeded at 3×10^5 cells per dish in 1 ml of Eagle minimal essential medium on sterile glass cover slips in 35-mm petri dishes together with an equal number of unlabeled, uninfected cells. Reciprocal mixtures were set up in which the uninfected cells were labeled, and controls of unmixed cells of each type were similarly set up on cover slips at 6×10^5 cells per dish. After 6 h in a 95% air–5% CO_2 incubator at 37°C, the cover slips were gently washed with phosphate-buffered saline, fixed dropwise in a fresh mixture of chilled absolute methanol plus glacial acetic acid (3:1), air dried, and then mounted with diluted Permount (Fisher Scientific) on clean slides, cell side up. The slides were dipped in Kodak nuclear tract emulsion NTB2 diluted 1:1 with water, dried and stored in light-tight slide boxes containing Drierite (Fisher Scientific) at 4°C for 10 days, and then developed in Dektol (Eastman Kodak) at 13°C. They were then fixed, cleared, washed, stained with Giemsa at pH 6.8, and air dried.

RESULTS

Enhancement of MuLV-induced fusion of murine cells by amphotericin B. The qualitative nature of the effect of Fungizone on retrovirus-induced fusion of cells is indicated in Fig. 1. In this experiment, plates containing ecotropic Moloney MuLV-infected NIH 3T3 cells at approximately 50% confluency were overlaid with an equal number of uninfected murine cells, in both the presence and the ab-

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TABLE 1. Cell lines used in this study

Cell line	Species and type	Virus type	Reference
$B_5$59	Mouse melanoma	Uninfected	46
NIH 3T3	Mouse fibroblast	Uninfected	16
SC-1	Mouse fibroblast	Uninfected	12
BALB/3T3 cl A31	Mouse fibroblast	Uninfected	2
BALB/11A	Mouse, transformed fibroblast	Murine sarcoma virus nonproducer	1
FLV cl.57/NIH 3T3	Mouse fibroblast	Ecotropic Friend MuLV	32
69E5/SC-1	Mouse fibroblast	Ecotropic AKR MuLV	19
LB-5/SC-1	Mouse fibroblast	Ecotropic Moloney MuLV subclone	S. Silagi, unpublished results
WN1802-N/SC-1	Mouse fibroblast	Ecotropic BALB MuLV	31
WN1802-B/SC-1	Mouse fibroblast	Ecotropic BALB MuLV	31
RLV/NIH-3T3	Mouse fibroblast	Ecotropic Rauscher MuLV	50
MoLV Cl 1/NIH-3T3	Mouse fibroblast	Ecotropic Moloney MuLV	11
MoLV V11/JLS-V9	Mouse fibroblast	Defective Moloney MuLV	5
RL♀ 2.3/SC-1	Mouse fibroblast	Ecotropic BALB MuLV	10
RL♀ 8.1/SC-1	Mouse fibroblast	Ecotropic BALB MuLV	10
MCF-247/SC-1	Mouse fibroblast	Dualtropic AKR recombinant	14
FrMCF-1/NIH 3T3	Mouse fibroblast	Dualtropic Friend recombinant	47
RMCF -1/SC-1	Mouse fibroblast	Dualtropic Rauscher recombinant	49
R-XC$^-$/NIH 3T3	Mouse fibroblast	Dualtropic Rauscher recombinant	50
Ampho 4070/NIH 3T3	Mouse fibroblast	Amphotropic MuLV	13
XC	Rat, transformed fibroblast	Rous sarcoma virus nonproducer	20

sence of Fungizone, and after overnight culture the plates were photographed under phase contrast. Although in the absence of the drug only XC cells were observed to undergo extensive fusion, in the presence of Fungizone all uninfected murine cell lines tested fused efficiently. There was no apparent correlation between oncogenic or morphological transformation and ease of fusion, as both normal untransformed fibroblasts, such as NIH 3T3, BALB/3T3 clone A31, and SC-1 cells, and transformed lines, such as clone 11A, a helper-free murine sarcoma virus-transformed subline of BALB/3T3, and B16 melanoma clone $B_5$59, were readily fused upon cocultivation with ecotropic MuLV-infected cells (Table 1; Fig. 1). The background level of fusion in homogeneous cultures containing only infected or uninfected cells was extremely low, even in the presence of Fungizone. Fusion was not observed when MuLV-infected cells were cocultivated with nonrodent cells, including cells of mink, dog, cat, and human origin (data not shown).

Fungizone contains two major components, the antibiotic amphotericin B and the detergent deoxycholate, which is used to maintain the antibiotic in solution. To determine which of these components was active in inducing the fusion, we examined solutions containing the individual reagents at concentrations equivalent to that present in the Fungizone solutions. At the concentrations tested deoxycholate did not stimulate fusion, whereas pure amphotericin B was as active as Fungizone in inducing fusion. The fusion-stimulating effect of amphotericin B is concentration dependent, with increasing fusion observed at concentrations up to 2 μg/ml and with a plateau at higher concentrations of the drug (Fig. 2). Interestingly, this plateau closely corresponds to the recommended fungicidal concentration of the drug (2.5 μg/ml). At this concentration, the level of fusion obtained was almost 3 orders of magnitude greater than that observed in control samples not containing the drug. This concentration was used for the studies described below.

Participation of both infected and uninfected cells in polykaryocyte formation. To determine the cellular origin of the nuclei in the syncytia formed upon cocultivation of infected and uninfected cells, ecotropic LB_5 MuLV-infected cells were cultured overnight in the presence of [^{3}H]thymidine, so that virtually all the cells were labeled, and cultivated with an equal number of unlabeled, uninfected SC-1, XC, or $B_5$59 melanoma cells. Autoradiography was performed as described in Materials and Methods. Two types of syncytia were detected: homokaryons, containing only uninfected nuclei, and heterokaryons, composed of both infected and uninfected cells (Fig. 3). Since the participation of infected cells in MuLV-induced polykaryocytes had not previously been noted, the extent of this phenomenom was quantitated. When labeled LB_5 MuLV-infected SC-1 cells were used, a mixture of both labeled and unlabeled nuclei was found in 46% of the syncytia formed with unlabeled SC-1 as the uninfected parent, in 86% of the syncytia with unlabeled XC cells, and in 27% of the syncytia with unlabeled $B_5$59 cells. An analysis of the ratio of labeled to unlabeled nuclei in the heterokaryons formed between infected and uninfected SC-1 cells indicated that approximately 30% of the nuclei came from infected cells (Table 2). Similar results were obtained when uninfected SC-1 cells were used as the labeled parent. These results indicate that cell-cell fusion between infected and uninfected cells occurs efficiently under these conditions and contributes significantly to the level of polykaryocyte formation.

Restriction of efficient fusion to ecotropic MuLVs. Three distinct classes of MuLVs which can efficiently infect murine cells have been identified: ecotropic, dualtropic, and amphotropic. To determine the specificity of the fusion reaction with respect to these classes, a series of representatives of each virus class were tested for their ability to fuse uninfected SC-1 cells in the presence of Fungizone. Only ecotropic virus-infected cells were positive, and different ecotropic virus-infected cells varied in their fusion activity (Table 3). The highest levels of fusion were observed with cells infected with two Moloney virus isolates, intermediate levels were observed with several other endogenous ecotropic viruses, and the cloned isolates of Friend and Rauscher ecotropic viruses tested were considerably less fusogenic. The efficiency with which these viruses fused SC-1 cells in

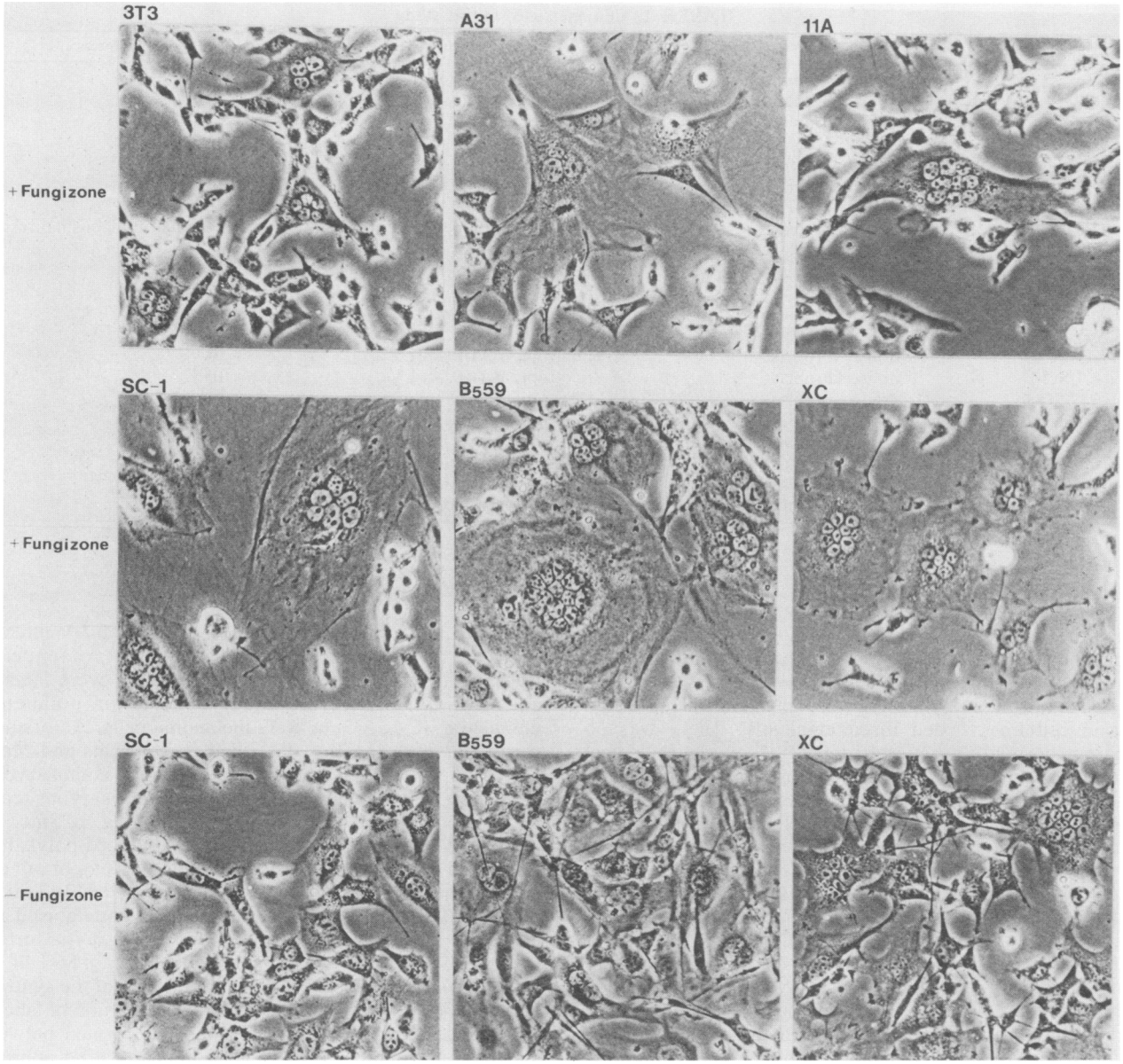

FIG. 1. Photomicrographs of polykaryocytes formed upon cocultivation of uninfected rodent cell lines with Moloney cl.1-infected NIH 3T3 cells. Representative areas showing typical polykaryocytes were photographed under phase contrast at 21 h after mixing of cells. Cells were all grown in Dulbecco modified minimal essential medium supplemented with 5% calf serum and 5% fetal calf serum. Fungizone (GIBCO) at a final concentration of 2.5 μg/ml was included in the medium of the samples so indicated.

these cocultivation assays was proportional to their efficiencies in fusing XC cells under standard conditions.

In contrast to the results obtained for ecotropic viruses, cells infected with dualtropic, amphotropic, or xenotropic viruses did not induce appreciable levels of fusion upon cocultivation with mouse cells, even in the presence of amphotericin B (Table 3). Small polykaryocytes were occasionally detected upon cocultivation of SC-1 cells with cells infected with some dualtropic viruses, but the extent of fusion was never above 2% of that observed for the most efficiently fusing ecotropic viruses. Since the major structural differences between the dualtropic gp70s and their ecotropic parents reside in the amino-terminal domain of the molecule, this suggests a role for this region of the gp70 protein in fusion.

A role for budding virions in the observed fusion is suggested by the observation that although cells producing Moloney ecotropic virus efficiently fused with SC-1 cells, fusion did not occur with cells infected with the Moloney-related mutant V11. The V11-infected cells produce equally high levels of ecotropic envelope proteins, but are deficient in *gag* protein synthesis and in assembly and release of virus particles (5). The inability of these cells to induce XC cell fusion has previously been reported (5).

Identification of two specific sites on gp70 involved in fusion. Studies of MuLV-induced fusion of XC cells have indicated a mechanistic role for the gp70 envelope protein (53). To determine the roles of the different MuLV *env* proteins in the fusion of murine cells, we examined the ability of monospecific antisera and monoclonal antibodies to MuLV *env* proteins gp70 and p15(E) to neutralize the fusion reaction between ecotropic MuLV-infected and uninfected cells. The

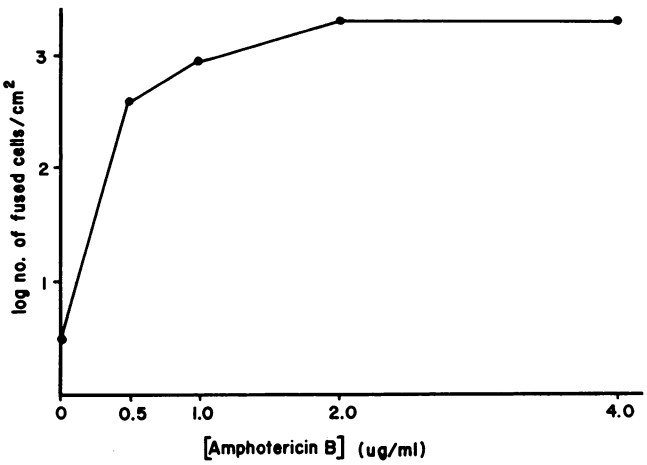

FIG. 2. Concentration dependence of amphotericin B enhancement of fusion. Equivalent numbers of LB_5-infected and uninfected SC-1 cells were plated together in medium containing the indicated concentrations of amphotericin B, and after 20 h the plates were fixed and stained, and the number of fused cells was determined. Amphotericin B dilutions were prepared from a stock solution of the drug in dimethyl sulfoxide at 5 mg/ml. Parallel plates containing equivalent concentrations of dimethyl sulfoxide did not exhibit increased levels of fusion over that of the control.

TABLE 3. Quantitation of fusion of SC-1 cells upon cocultivation with MuLV-infected mouse fibroblasts[a]

Virus type	Virus/cell	10^2 No. of fused cells/cm²
Ecotropic viruses	LB_5/SC-1	118
	MoLV/NIH 3T3	70
	RL♀ 2.3/SC-1	67
	WN1802B/SC-1	66
	RL♀ 8.1/SC-1	43
	WN1802N/SC-1	41
	69E5/SC-1	32
	RLV/NIH 3T3	11
	FLV cl.57/NIH 3T3	7
Ecotropic nonproducer mutant	MoV11/JLSV9	<0.1
Nonecotropic viruses	MCF-247/SC-1	2
	FrMCF-1/NIH 3T3	1
	RXC^-/NIH 3T3	1
	RMCF-1/SC-1	<0.1
	Ampho 4070/NIH 3T3	<0.1

[a] Monolayers of SC-1 cells at 25% confluency were overlaid with an equivalent number of infected cells in medium containing Fungizone and incubated for 24 h. After fixing and staining, the extent of fusion was quantitated.

results of these experiments indicate that antisera directed against gp70 efficiently inhibited fusion, whereas an antiserum and several monoclonal antibodies directed against different regions of p15(E) (35) did not inhibit fusion (Table 4). For a series of monoclonal antibodies directed against seven distinct determinants of gp70, only two were found to possess significant fusion-inhibiting activity: antibody 35/56, directed against epitope f, and antibody 35/299, directed against epitope g (37). A quantitative comparison of the activities of the two active and one inactive rat monoclonal anti-gp70 antibodies indicated that antibodies 35/56 and 35/299 possess similar fusion inhibitory activities and give similar titration curves (Fig. 4). This result is of interest because the epitopes reacting with these two antibodies are located on separate domains of the gp70 molecule (37) and because other antibodies recognizing determinants on these domains do not exhibit significant fusion-inhibiting activity. This suggests that the two epitopes recognized by these antibodies may specifically play roles in the reactions leading to fusion.

Sensitivity of fusion to interference by endogenous expression of ecotropic gp70. The sensitivity of MuLV-induced fusion of mouse cells to interference by endogenously expressed viral proteins was examined. Cells infected with ecotropic MuLVs of either the AKR or FMR type, including defective Moloney mutant V11, were totally resistant to fusion with LB_5-infected SC-1 cells, whereas cells infected with dualtropic or amphotropic viruses fused extensively (Table 5). These results suggest that sensitivity to fusion requires the presence of free ecotropic-specific receptors on the target cells, which are blocked by the endogenous expression of ecotropic but not nonecotropic envelope proteins. This suggests a simple assay for classifying ecotropic viruses. For this purpose a cell line expressing an efficiently fusing MuLV, such as Moloney, can be cocultivated in the

TABLE 2. Numbers of nuclei of uninfected and LB_5 MuLV-infected SC-1 fibroblasts in heterokaryons[a] formed 6 h after being mixed in the presence of Fungizone

[³H]thymidine labeled cells[b]	No. (%)[c] of SC-1 nuclei	No. (%)[c] of LB_5/SC-1 nuclei
LB_5/SC-1	512 (68)	242 (32)
SC-1	67 (70)	29 (30)
Total	579 (68)	217 (32)

[a] Heterokaryons are polykaryocytes containing nuclei from both infected and uninfected parental cells.

[b] Cells were seeded and prepared for autoradiography as described in Materials and Methods. Labeled and unlabeled nuclei were counted in all heterokaryons with four or more nuclei.

[c] Numbers in parentheses represent percentage of total nuclei present in heterokaryons.

TABLE 4. Inhibition of fusion by monoclonal antibodies and hyperimmune sera directed against MuLV *env* proteins[a]

Antibody	Specificity[b]	Titer (50% inhibition of fusion)
GoatαR gp70	gp70	1:4,000
GoatαBV2 gp70	gp70 C-term	1:8,000
35/56	$gp70^f$	>1:32,000
35/299	$gp70^g$	>1:8,000
42/94	$gp70^h$	<1:50
19-A2	$gp70^c$	<1:50
16-C1	$gp70^b$	<1:50
19V-E5	$gp70^e$	<1:50
16-E4	$gp70^d$	<1:50
Rabαp15(E)	p15(E)	<1:50
9-E8	$p15(E)^a$	<1:50
42/114	$p15(E)^c$	<1:50

[a] Akv/SC-1 cells were mixed with the appropriate dilutions of antisera in Fungizone-containing medium and added to plates containing uninfected cells. After cocultivation for 20 h, the cells were fixed and stained, and the extent of fusion was quantitated.

[b] Superscripts represent specific epitopes of gp70 and p15(E) as defined in reference 37.

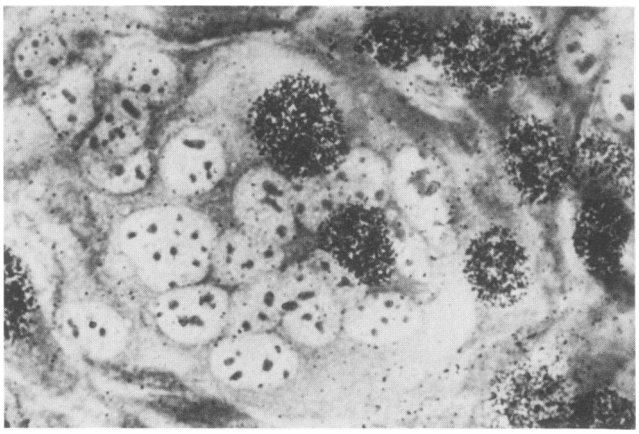

FIG. 3. Typical polykaryocyte formed between radioactively labeled LB_5-infected SC-1 cells and uninfected SC-1 cells after cocultivation for 6 h. In this experiment, a 10-fold ratio of unlabeled to labeled cells was used. Two infected nuclei, together with more than a dozen uninfected nuclei, are present in the illustrated polykaryocyte.

presence of Fungizone with SC-1 or 3T3 cells infected with the virus in question, and the extent of fusion can then be quantitated. Lack of fusion would indicate the presence of an ecotropic virus in the target cells. This indirect interference assay should efficiently identify ecotropic viruses which themselves may have low fusion efficiency.

DISCUSSION

In this paper we report that fusion between ecotropic MuLV-infected and uninfected murine cells is greatly enhanced by the presence of amphotericin B in the culture medium. Amphotericin B binds to cholesterol in biological membranes (23) and increases the permeability of cells (26, 27). Changes in membrane fluidity resulting from interactions between amphotericin B and cholesterol in the plasma membrane are believed to play a role in the enhancement of Sendai virus-mediated fusion by this drug (18). A similar effect may be involved in the enhancement of MuLV-induced fusion.

The MuLV-induced fusion of mouse cells that occurs in the presence of amphotericin B appears to be mechanistically analogous to the previously reported fusion of XC cells by these viruses (20). Rapid MuLV-mediated fusion of XC cells occurs in the presence of inhibitors of protein and nucleic acid synthesis (17, 53), indicating that viral replication in the target cells is not required for fusion and suggesting that this is an example of "fusion from without" (40). Efficient fusion of murine cells in the presence of amphotericin B is observed as early as 3 h after mixing of infected and uninfected cells, suggesting that in this case too, de novo viral replication is not required for polykaryocyte formation. Efficient fusion by virus alone was not observed under normal conditions of cell-free infection, but did occur when concentrated virions were used (data not shown). Autoradiographic analysis of the syncytia formed upon cocultivation of infected and uninfected cells indicates that significant fusion occurs between these cells (Fig. 3; Table 2). This implies that cell-associated viral components can interact with appropriate receptors on uninfected cells, leading to fusion. Of interest is the fact that cells infected with Moloney V11, a mutant which expresses high levels of envelope proteins on the cell surface, but which does not produce any budding viral particles (5), do not undergo fusion. This suggests that cell-associated viral components may acquire their fusogenic capacity at the stage of the budding particle.

Our analysis of the effect of the fusion-inhibiting activities of various antibodies directed against the MuLV *env* proteins demonstrates the presence of two separate sites on gp70 which are involved in fusion (Table 4; Fig. 4). The inability of antibodies to p15(E) to inhibit fusion is consistent with results previously obtained for MuLV-induced fusion of XC cells (53) and indicates that the regions of p15(E) bearing the major antigenic sites of the protein are not involved in fusion. The two anti-gp70 antibodies with neutralizing activity were previously shown to react with epitopes located on different ends of the gp70 molecule: 35/299 reacted with an amino-terminal site, and 35/56 reacted with a carboxy-terminal site (37). The amino-terminal domain distinguishes mink cell focus-forming virus gp70s from their ecotropic parents and thus presumably contains the receptor-binding site of these molecules (6, 15, 21, 36). The difference in fusogenicity of ecotropic and dualtropic MuLVs suggests that this domain is important for fusion; this is confirmed by the efficient inhibition of fusion by antibody 35/299. The fusion-inhibitory activity of antibody 35/56 demonstrates that the C-terminal domain of gp70 possesses a functional role in fusion as well. The epitope recognized by this antibody has previously been correlated with the G_{IX} site (34), and there is evidence correlating the G_{IX}^+ phenotype with the presence of an aspartic acid residue at position 404 from the amino terminus of gp70 (9, 25, 45). This residue immediately follows a stretch of 27 uncharged, mostly hydrophobic amino acids and is 36 residues away from the hydrophobic N terminus of the p15(E) protein in the uncleaved *env* precursor. This suggests that either one or both of these hydrophobic domains of the *env* proteins may play a critical role in fusion, in analogy with the demonstrated role of the hydrophobic N-terminal domain of the F protein in paramyxovirus-induced fusion (43, 44). The fusion-inhibitory activity of antibody 35/56 may correlate with the potent virus-neutralizing activity of this antibody previously described (30).

Syncytium formation is a common feature of a large number of enveloped viruses and is believed to be mediated by mechanisms in common with the process of virus-cell fusion which occurs during the infectious process. Two separate pathways have been described for viral infection, which differ in the site and pH optimum for fusion. For influenza virus, vesicular stomatitis virus, and several

TABLE 5. Effect of MuLV expression on ecotropic virus-induced fusion[a]

Target cells	10^2 Fused cells/cm^2
FLV cl.57/NIH 3T3	<1
LB_5/SC-1	<1
69E5/SC-1	<1
MoV11/JLSV9	<1
FrMCF-1/NIH 3T3	120
R-XC$^-$/NIH 3T3	176
Ampho 4070/NIH 3T3	256

[a] Monolayers of LB_5-infected SC-1 cells were overlaid with an equivalent number of infected cells in the presence of Fungizone, and the extent of fusion was quantitated after 20 h.

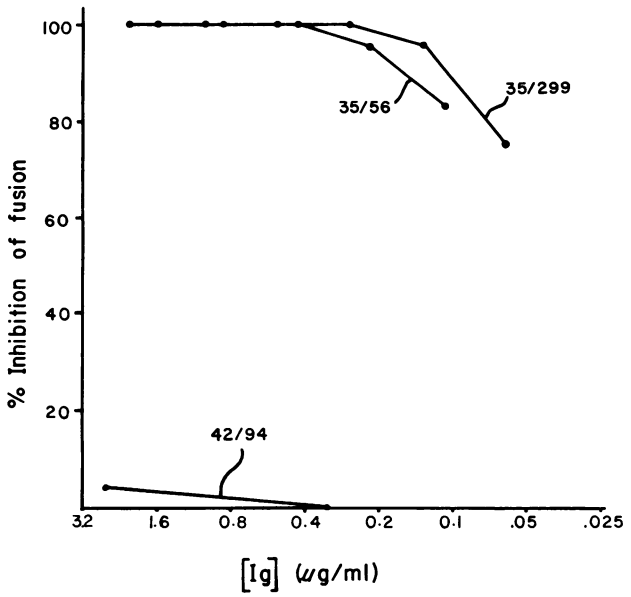

FIG. 4. Titration of fusion-inhibiting activity per microgram of antibody for three monoclonal antibodies directed against different epitopes of ecotropic Akv gp70. All three antibodies are of rat origin and of the immunoglobulin G1 subclass. Immunoglobulin concentrations were determined with radial immunodiffusion plates (Miles); rat sera with known immunoglobulin concentrations were used as standards.

togaviruses, fusion requires a pH below 6; these viruses are believed to enter cells by endocytosis followed by fusion between viral and endocytic or lysosomal membranes (48, 51). The recent description of the efficient fusion of mouse mammary virus-infected cells after exposure to conditions of low pH suggests that this virus also utilizes this pathway (42). Paramyxoviruses, on the other hand, fuse efficiently under neutral conditions and appear to be capable of penetration at the plasma membrane (28). Previous studies of the pathway utilized during MuLV-induced fusion were not conclusive. It was reported that infection by MuLV is inhibited by lysomotropic bases (4, 33) and that internalized gp70 is rapidly degraded (3), consistent with the endocytic pathway. On the other hand, it has recently been shown that fusion of *M. dunni* cells by Friend MuLV is optimal at pH 7.6 and is markedly inhibited at pH < 6.4 (39). Our demonstration, in the work described in this paper, of efficient MuLV-induced fusion of mouse cells under neutral conditions is consistent with the latter results and shows that under appropriate conditions fusion at the plasma membrane can occur for MuLV. Further studies are required to elucidate the relative importance of such fusion during infection.

The enhancing effect of amphotericin B on MuLV-induced fusion of mouse cells should be useful in further mechanistic studies of the role of specific viral components in the fusion reaction, and it provides an efficient assay which may facilitate the identification and characterization of the cell surface receptor molecules which interact with the viral envelope proteins during the processes of infection and syncytium formation.

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