Cationic Liposomes (Lipofectin) Mediate Retroviral Infection in the Absence of Specific Receptors

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We have used cationic liposomes (Lipofectin) to facilitate retrovirus infection of cells lacking the homologous viral receptor. Ecotropic murine leukemia virus and packaged retroviral vectors were shown to infect mink cells, and amphotropic packaged retroviral vectors were shown to infect hamster cells in the presence of Lipofectin but not in the presence of Polybrene. Lipofectin-mediated infection of cells lacking the homologous receptor results in a titer approximately 0.1% of the titer in cells with the homologous receptor, using the standard Polybrene protocol. The use of Lipofectin may provide a simple means to experimentally infect a wide variety of cells with viruses not normally infectious for the species, tissue, or cell type of interest.

One of the major determinants of viral host range specificity and cell tropism involves the interaction of surface components of virions with specific receptors on host cell membranes. Retroviruses are enveloped viruses that have surface glycoproteins (generically designated SU) which interact with these receptors. A major classification scheme of murine leukemia virus (MuLV) is based on the host range determined by the specific SU (gp70) (i.e., ecotropic, xenotropic, and amphotropic or polytropic) and the corresponding species-specific or shared receptors (for a review, see reference 36).

The first step in viral infection is binding to the homologous receptor, followed by penetration and uncoating (for reviews, see references 2 and 31). Viral receptors avidly bind virions; however, their role in virus penetration is not well defined. Enveloped animal viruses are thought to enter cells by one of two major pathways, either direct fusion between the envelope of the virus and the cell membrane or endocytosis (for a review, see reference 38). Most of the recent studies with retroviruses favor a direct fusion mechanism (17, 19, 20, 29, 40).

Retrovirus infectivity is generally undetectable in cells that lack specific receptors. Infectivity is greatly reduced if receptors are saturated because of the production of envelope glycoprotein by an endogenous virus or *env* gene or by infection with an exogenous virus (13, 24, 25, 27, 28). Conversely, retroviruses defective in the synthesis of envelope glycoprotein produce virions which are incapable of interaction with these receptors and are consequently not infectious (8, 26).

Various experimental strategies have been used to facilitate virus infection in situations in which either the SU glycoprotein or the membrane receptor was missing. UV-inactivated Sendai virus, historically used as a reagent to fuse cell membranes, can mediate fusion between cell membranes and the glycoprotein-deficient (noninfectious) envelope of the Bryan high-titer strain of Rous sarcoma virus, resulting in infection (9, 37). Polyethylene glycol, another reagent capable of nonspecifically fusing membranes, has been shown to facilitate Rous sarcoma virus infection of cells lacking receptors and also allows the Bryan high-titer strain of Rous sarcoma virus to become infectious (22).

Liposomes have been used to encapsulate and deliver to cells a variety of materials, including drugs (for a review, see reference 7), nucleic acids (for reviews, see references 15 and 30), and viral particles (4, 12, 39). Traditional methods of producing liposomes (i.e., reverse-phase evaporation vesicles or large unilamellar vesicles produced by calcium-EDTA chelation) are laborious, and the harsh treatments cause considerable loss of the biological activity of viruses (4). We examined the utility of the recently described cationic liposomes, Lipofectin (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) (5, 6), as a means to facilitate retrovirus infection in the absence of specific receptors. Lipofectin spontaneously complexes with DNA and RNA in solution and facilitates fusion of the complex with cells in culture, resulting in the efficient transfer of nucleic acids to a wide variety of eucaryotic cell types.

Infection of nonpermissive mink cells with ecotropic virus or vector. Preliminary experiments demonstrated that heterologous cells not susceptible to ecotropic MuLV (i.e., lacking the specific receptor) could be infected in the presence of Lipofectin. The effective concentration range was determined to be between 4 and 16 μg/ml. Preincubation of virus in medium with Lipofectin for 1 to 60 min at room temperature or on ice prior to infection of nonpermissive cells resulted in similar titers (data not shown). Likewise, exposure of the cells to the virus-Lipofectin complex for 2 to 18 h resulted in similar titers. Exposures of less than 2 h were less effective (data not shown).

To quantitatively demonstrate the effectiveness of Lipofectin in allowing retrovirus infection in nonpermissive cells, we exposed CCL 64 mink lung fibroblasts (11) to the ecotropic packaged retroviral vector RV-neo(N-Pac) (1) in the presence of Lipofectin or Polybrene (14, 34) or in the absence of a polycation for 2 h followed by refeeding in routine maintenance medium. Selection for G418-resistant colonies began the day after infection, and colonies were stained on day 15 for counting. Permissive AKR mouse cells
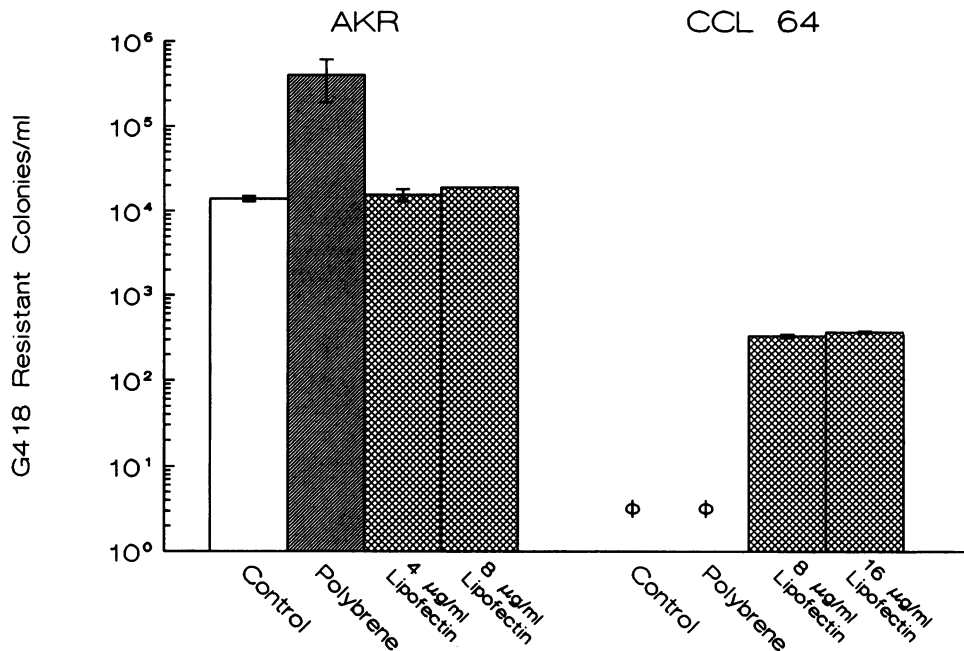

FIG. 1. Infection of AKR mouse and CCL 64 mink cells with ecotropic RV-neo(N-Pac). Control is exposure to virus in the absence of polycation; the Polybrene concentration is 16 μg/ml, and the Lipofectin concentration is indicated. φ, No colonies were detected with 1 ml of undiluted, packaged vector stock. The G418-resistant colony counts of duplicate wells (or more in some cases) were averaged. Bars indicate standard deviations. The absence of bars indicates that duplicate colony counts were identical.

were included for comparison. G418-resistant CCL 64 colonies were not observed following addition of virus either in the presence of Polybrene or in the absence of polycation (Fig. 1). However, in the presence of Lipofectin (8 or 16

μg/ml), approximately 3 × 10² CFU was observed, which is approximately 0.1% of the titer of this virus stock in AKR cells (permissive for ecotropic MuLV), using Polybrene (Fig. 1). Preliminary experiments suggested that the pres-
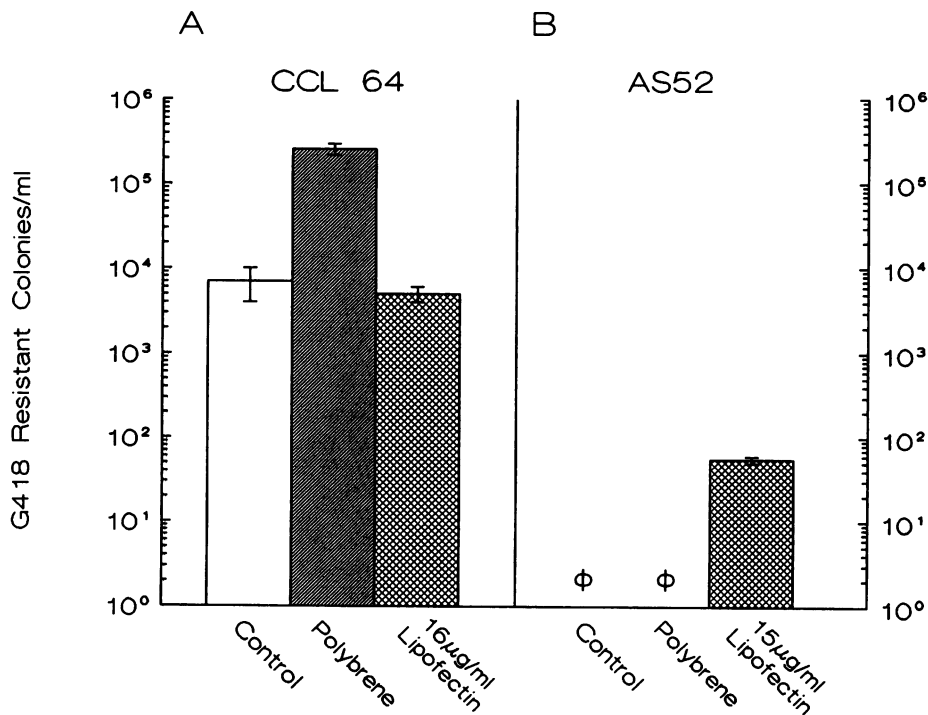
FIG. 2. Infection of permissive and nonpermissive heterologous cells with amphotropic RV-neo(PA317). (A) CCL 64 mink cells were infected and assayed as described in the legend to Fig. 1. (B) AS52 hamster cells were infected and assayed as described in the legend to Fig. 1, except that colonies were stained on day 10.

A   B
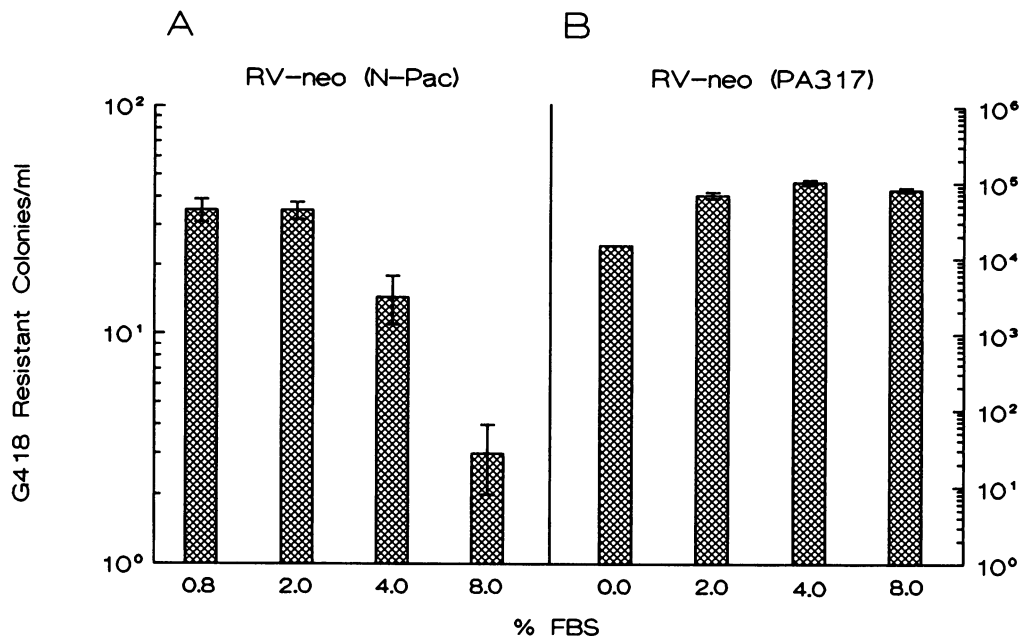
RV–neo (N–Pac)   RV–neo (PA317)

FIG. 3. Effect of FBS on Lipofectin with nonpermissive and permissive infection. (A) CCL 64 cells were infected with a 1:10 dilution of RV-neo(N-Pac), in the presence of 8 μg of Lipofectin per ml in the indicated final concentration of FBS. (B) CCL 64 cells were infected with dilutions of RV-neo(PA317) in the presence of 8 μg of Lipofectin per ml in the indicated concentration of FBS. Samples marked 0.0% serum actually contain 0.008% FBS residual from the virus stock (i.e., a 10^{-3} dilution of the viral stock containing 8% FBS). G418-resistant colonies arising in duplicate dishes were quantitated and averaged.

ence of Polybrene abolishes the effect of Lipofectin in nonpermissive cells, probably by competing with Lipofectin for binding viral envelopes or cell membranes. The titer in AKR cells without a polycation enhancer was lower than that in cells with Polybrene, as expected. The titer in AKR cells with Lipofectin was approximately the same as that in the control, suggesting that under these conditions the cationic property of Lipofectin does not substitute for Polybrene in enhancing permissive infectivity. It seems likely that the fusogenic property of Lipofectin is irrelevant for infection in permissive cells in which virus attachment and penetration utilize the appropriate receptor.

Six independent G418-resistant CCL 64 colonies were isolated and expanded. These clones were assayed for the ability of the RV-neo vector to be rescued by infection with the amphotropic MuLV (4070A). The RV-neo vector was rescued from all six G418-resistant cell lines by 4070A MuLV as determined by conferring G418 resistance to mouse SC-1 cells (data not shown). These data indicate that the RV-neo vector is integrated and expressed in a retrovirus-specific manner in these CCL 64 clones infected by a Lipofectin-mediated process.

Additional experiments utilizing a modification of the XC plaque assay (23) demonstrated that CCL 64 cells could be infected by Moloney MuLV in the presence of Lipofectin (8 μg/ml) with an infectivity of approximately 0.1% of the titer in SC-1 cells. Again, no infectivity was observed in the presence of Polybrene.

Infection of permissive and nonpermissive heterologous cells with amphotropic vectors. We examined the effects of Lipofectin on permissive viral infection in mink cells, using RV-neo packaged by the amphotropic packaging cell line PA317 (18). CCL 64 cells were infected with RV-neo(PA317) under the three conditions of the experiment shown in Fig. 1. Infection in the presence of Lipofectin was equivalent to that

of the control, while Polybrene enhanced infectivity almost 100-fold (Fig. 2A). In accord with results from the permissive combination of ecotropic virus and AKR cells (Fig. 1), amphotropic viral infection of mink cells is apparently unaffected by Lipofectin. Similar results were observed with amphotropic virus infection of AKR cells (data not shown).

Chinese hamster cells are not susceptible to infection by standard ecotropic or amphotropic MuLV isolates (10, 21), thus excluding several important cell lines from retrovirus-mediated gene transfer. AS52 cells are hypoxanthine phosphoribosyltransferase-deficient Chinese hamster ovary (CHO) cells that carry a single functional copy of the bacterial *gpt* gene stably integrated into the CHO genome (32, 33). AS52 cells were exposed to RV-neo(PA317) under the three conditions shown in Fig. 1. In the presence of Lipofectin but not in the presence of Polybrene or the absence of polycation, AS52 cells were susceptible to infection by RV-neo(PA317) (Fig. 2B). These data are comparable to the results obtained by using the ecotropic packaged vector RV-neo(N-Pac) to infect nonpermissive mink cells (Fig. 1).

Effects of serum on Lipofectin-mediated infection. It has been reported that serum inhibits the DNA transfection efficiency of Lipofectin (5). We have observed that undiluted medium harvested from virus-producing cells (containing 8% fetal bovine serum [FBS]) yields fewer infectious units than does a 1:10 dilution (containing 0.8% FBS). Experiments to assess the effect of serum on Lipofectin-mediated infection were performed by infecting CCL 64 cells with RV-neo(N-Pac) in the presence of 0.8% (residual in the 1:10 dilution of virus stock) to 8.0% FBS. The data in Fig. 3A demonstrate a concentration-dependent inhibition of Lipofectin-mediated infectivity by serum.

In contrast, permissive infectivity in the presence of Lipofectin was slightly enhanced by serum (Fig. 3B). Thus,

the data presented in Fig. 1 and 2 suggesting that permissive infections are unaffected by Lipofectin are valid only in the absence of serum. Lipofectin may have some detrimental effects on virus or cells which are modulated by the presence of serum, thereby allowing the cationic property of this reagent to enhance infectivity in a manner similar to that of Polybrene and other polycations (3, 14, 34, 35). Neither Polybrene-enhanced infection or infection in the absence of a polycation was affected by the addition of comparable concentrations of serum (data not shown).

Lipofectin is a preformed cationic liposome which is thought to facilitate DNA transfection by forming a complex with nucleic acid which subsequently fuses with the cell membrane, resulting in the delivery of DNA (or RNA) to the cell (6). The fusogenic property of cationic liposomes is controlled, in part, by the choice of neutral phospholipid in the complex (5, 16). Since negatively charged nucleic acids are added to preformed, positively charged vesicles, it is thought that the DNA or RNA is bound to the vesicle surface rather than in the aqueous interior. We assume a similar structure for Lipofectin-virion complexes. With traditional liposomes, virions must be trapped in the aqueous interior in order to infect cells lacking specific receptors (39). We speculate that Lipofectin fuses with both cell membrane and viral envelope, delivering a functionally active virion core into the cytoplasm.

Notably, avian retroviruses can also infect heterologous cells in the presence of Lipofectin (J. Olsen and R. Swanstrom, personal communication). The practical value of the Lipofectin-mediated infection is to conveniently extend the host range of experimental virus infections and to superinfect cells normally resistant because of homologous interference. For example, by using Lipofectin, extending the host range of avian retrovirus vector packaging systems to mammalian cells or allowing retrovirus vectors to superinfect chronically infected cells may be considered. In addition, certain virus isolates may lose their infectivity because of unstable envelopes. Lipofectin may provide a way to restore infectivity, especially for clinical samples, aiding in diagnoses or providing a means for further experimentation. Also, Lipofectin-mediated infection is likely to extend to viruses other than retroviruses. Finally, this technique may also be useful in experiments designed to better explain normal viral adsorption and entry.

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