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Enhancement of human immunodeficiency virus type 1 infection by cationic liposomes: the role of CD4, serum and liposome–cell interactions

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We have reported previously the enhancement of the infectivity of human immunodeficiency virus type 1 (HIV-1) by liposomes composed of the cationic lipid *N*-[2,3-(dioleyloxy) propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA). To determine the mechanism by which this process occurs, we have investigated the role of CD4, serum concentration and liposome–cell interactions in the DOTMA-mediated stimulation of HIV-1 infection of A3.01 cells. Serum alone significantly inhibited the binding and infectivity of HIV-1, but DOTMA-mediated enhancement of infectivity was more pronounced in the presence of serum than in its absence. HIV-1 binding to cells was increased in the presence of DOTMA liposomes, DEAE-dextran and polybrene, all of which also enhanced infectivity to a similar extent at comparable concentrations. Fluorescence dequenching measurements indicated that DOTMA liposomes fused with HIV-1, but not with cell membranes, in the presence of serum. The enhancing effect of DOTMA liposomes on HIV-1 infectivity was CD4-dependent, and appeared to involve virus–liposome fusion and liposome binding to the cell surface. DOTMA liposomes did not mediate infection of the CD4$^-$ K562 and Raji cell lines.

Introduction

Human immunodeficiency virus type 1 (HIV-1) is known to use the CD4 protein as its cellular receptor, and to infect CD4$^+$ T lymphocytes (Dalgleish *et al.*, 1984; Klatzmann *et al.*, 1984; McDougal *et al.*, 1986) and other CD4-bearing cells, such as monocytes and macrophages (Gartner *et al.*, 1986; Ho *et al.*, 1986; Nicholson *et al.*, 1986), Langerhans cells (Tschachler *et al.*, 1987) and human peripheral blood dendritic cells (Patterson & Knight, 1987). A number of studies have also shown that cells that do not express demonstrable amounts of CD4 can be infected with HIV, although at low levels (Cheng-Mayer *et al.*, 1987; Dewhurst *et al.*, 1987; Clapham *et al.*, 1989; Harouse *et al.*, 1989; Tateno *et al.*, 1989; Li *et al.*, 1990; Cao *et al.*, 1990; Konopka *et al.*, 1991). That infection is not prevented by anti-CD4 antibodies or neutralizing doses of recombinant soluble CD4 (rsCD4) (Clapham *et al.*, 1989; Weber *et al.*, 1989; Weiss *et al.*, 1989) suggests that receptors other than CD4 are responsible for the susceptibility of these cells to HIV. Expression of the CD4 molecule on the surface of certain cells by transfection does not render them susceptible to HIV-1 infection (Maddon *et al.*, 1986) or enhance infectivity (Chesebro *et al.*, 1990), suggesting that other membrane factors may be involved in the initial fusion step.

There are two essential steps in HIV-1 entry into the cell: binding of virus to the cell membrane, and fusion of viral and cell membranes. Both steps are mediated by two viral envelope proteins, a trans-membrane glycoprotein, gp41, thought to mediate the fusion step, and a glycoprotein, gp120, attached non-covalently to gp41, responsible for binding to CD4 (Lifson *et al.*, 1986*a*; Sodroski *et al.*, 1986; Kowalski *et al.*, 1987). It is thought that the high affinity binding of gp120 to CD4 (Lasky *et al.*, 1987) triggers a conformational change in gp120 that reveals a fusogenic domain of gp41 (Kowalski *et al.*, 1987, 1991; Bosch *et al.*, 1989). Binding of rsCD4 or the purified V1 domain of sCD4 to gp120 on HIV-1 virions results in dissociation of gp120 from its complex with gp41 (Moore *et al.*, 1990); thus, shedding of gp120 may represent the initial stage in virus–cell fusion.

Polycationic reagents such as polybrene or DEAE-dextran have frequently been used to increase the efficiency of retrovirus infection *in vitro* (Toyoshima &

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Vogt, 1969; Cheng-Mayer *et al.*, 1987; Chesebro & Wehrly, 1988; Zaia *et al.*, 1988; Tateno *et al.*, 1989; Busso & Resnick, 1990; Ikeuchi *et al.*, 1990). It has been suggested that the polycation-mediated enhancement is due at least in part to increased adsorption of the virus to the cell, but it may also facilitate virus penetration; however, the exact mechanisms by which infectivity is increased remain unclear. Infectivity is enhanced when target cells are pretreated with polycations or exposed to virus in the presence of the polycations, but not when virus is preincubated with the polycations (Toyoshima & Vogt, 1969).

We have reported recently that preincubation of HIV-1 with liposomes composed of the positively charged synthetic lipid *N*-[2,3-(dioleyloxy) propyl]-*N*,*N*,*N*-trimethylammonium chloride (DOTMA), or the addition of such liposomes during exposure of cells to the virus, enhances the infectivity of HIV-1 (Konopka *et al.*, 1990). Sensitivity of various cell types to DOTMA-mediated enhancement of HIV-1 infection correlates with the expression of CD4 on the cell surface (Konopka *et al.*, 1990).

Liposomes containing DOTMA can mediate the delivery of DNA (Felgner *et al.*, 1987) and RNA (Malone *et al.*, 1989) into cultured mammalian cells. Pure DOTMA liposomes have been shown to deliver a gene regulatory protein and protein–DNA complexes (Debs *et al.*, 1990) into such cells, although the mechanism of delivery is not completely understood. DOTMA liposomes interact with DNA molecules to form lipid–DNA complexes. It has been proposed that DOTMA–DNA complexes bind to the plasma membrane, fuse and deliver DNA into the cytoplasm (Felgner *et al.*, 1987; Felgner & Ringold, 1989; Malone *et al.*, 1989). The interaction (fusion) of DOTMA-containing liposomes with cells and subsequent transfection is inhibited by the presence of serum (Felgner *et al.*, 1987); this result may be explained by binding of DOTMA–DNA complexes to serum constituents.

In contrast, DOTMA liposomes enhance HIV-1 infectivity in the presence of 10% foetal bovine serum (FBS) (Konopka *et al.*, 1990). Therefore, it was of interest to determine the effect of serum on the enhancement of HIV-1 infectivity by DOTMA. Since we had postulated that the enhancement of infectivity could be due to fusion of HIV-1 with DOTMA liposomes, it was also necessary to examine whether serum affected this fusion process. Furthermore, to elucidate the mechanism of action of DOTMA-mediated enhancement of infectivity, we investigated the interaction of DOTMA liposomes with host cells, both in the absence and presence of serum. To determine whether the stimulation of infectivity in $CD4^+$ cells occurred using the CD4 receptor, we examined the role of soluble CD4 and anti-Leu3a antibodies in this process. Since the enhancement of HIV-1 infectivity by DOTMA liposomes raised the possibility of infecting $CD4^-$ cells by direct fusion with the plasma membrane bilayer, we investigated whether K562 and Raji cells could be infected in the presence of these liposomes.

Methods

Chemicals. DOTMA, synthesized as described previously (Felgner *et al.*, 1987), was kindly provided by N. Dyson at Syntex Research (Palo Alto, Ca., U.S.A.) and stored in ethanol under argon at −70 °C. Octadecylrhodamine (R_{18}) was purchased from Molecular Probes; *N*-(lissamine rhodamine B sulphonyl)-phosphatidylethanolamine (Rho-PE) was obtained from Avanti Polar Lipids; $C_{12}E_8$ detergent was from Calbiochem; rsCD4 was kindly provided by Genentech. Anti-Leu3a antibody was purchased from Becton Dickinson, DEAE–dextran was from Pharmacia and polybrene was from Sigma.

Cell lines. The $CD4^+$ human lymphoblastoid cell line, A3.01, was obtained from T. Folks (Centers for Disease Control, Atlanta, Ga., U.S.A.); the H9/HTLV-III_B cell clone was obtained from L. Arthur through the AIDS Research and Reference Reagent Program, NIAID; the $CD4^-$ human chronic myelogenous leukaemia cell line, K562, was obtained from ATCC; the $CD4^-$ human Burkitt's lymphoma B cell line, Raji, was obtained from Microbiological Associates through P. Maddon (NIH). Cells were cultured at 37 °C (7% CO_2) in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated FBS, penicillin (50 units/ml), streptomycin (50 μg/ml) and L-glutamine (2 mM).

Virus. The virus and infected cell lines were handled in P-2 and P-3 facilities as recommended by the Centers for Disease Control (1988). For fusion experiments, HIV-1 [LAV-1, obtained from the Pasteur Institute and provided by M. Jennings (University of California, Davis)] was propagated in human lymphoblastic leukaemia (CEM) cells purified as described (Larsen *et al.*, 1990) and stored at −70 °C. The virus preparation and the cell line tested negative for *Mycoplasma* contamination. A standard laboratory strain of HIV-1 (LAV) was originally provided by T.Folks and propagated in A3.01 cells. The culture supernatant was harvested at times of peak p24 production, filtered through a 0·22 μm Millipore filter, split into aliquots and stored at −80 °C. The reverse transcriptase activity of the virus stock solutions was $5{\cdot}1 \times 10^5$ and $8{\cdot}8 \times 10^5$ c.p.m./ml supernatant, as detected by the method of Hoffman *et al.* (1985). The yield of ELISA-reactive p24 protein in the virus supernatant was approximately 1·0 μg/ml. Virus inocula were standardized by their content of p24 antigen and cultures were infected with virus at a concentration of between 1 and 10 ng p24/10^6 cells.

Virus labelling. A fluorescence assay was used to monitor the mixing of virus and target membranes during fusion, using R_{18} as the fluorescent lipid marker (Hoekstra *et al.*, 1984; Loyter *et al.*, 1988; Larsen *et al.*, 1990). HIV-1 (100 μg) was labelled with a solution of R_{18} as described (Konopka *et al.*, 1990). The HIV-1 lipid:protein ratios reported previously (Aloia *et al.*, 1988) were assumed for determining the amount of R_{18} with which to label the virus.

Preparation of liposomes. Pure DOTMA liposomes were prepared in 150 mM-NaCl, 10 mM-TES (Sigma; Calbiochem) and 10 mM-sodium acetate pH 7·5 (fusion buffer). DOTMA liposomes for infectivity studies, and those containing a self-quenching concentration of Rho-PE (4 mol %) for binding and fusion studies, were prepared in NaCl/TES pH 7·5 by hydrating dried lipid (Bangham *et al.*, 1965) and extruding three times under argon through double polycarbonate

membranes of 0·08 or 0·2 μm pore diameter (Poretics) in a Lipex Biomembranes high pressure extrusion apparatus, to achieve a uniform size distribution of vesicles (Szoka *et al.*, 1980). This procedure results in the production of predominantly unilamellar vesicles (Düzgüneş *et al.*, 1983; Hope *et al.*, 1985).

Fusion experiments. The dilution of R_{18} or Rho-PE into the target membranes was measured by following the increase of rhodamine fluorescence, which results from the relief of self-quenching upon membrane fusion (Hoekstra *et al.*, 1984; Longe *et al.*, 1986; Düzgüneş & Bentz, 1988). Fluorescence measurements were performed with a Spex Fluorolog 2 fluorimeter or a Perkin-Elmer LS-5B fluorimeter (555 nm excitation, 595 nm emission and a 590 nm low end cut-off filter to minimize light scattering), linked through a MacAdios analogue:digital converter using Manager II software (GW Instruments) to a Macintosh SE/30 computer to collect raw data, as described earlier (Larsen *et al.*, 1990).

Binding of DOTMA liposomes to A3.01 and Raji cells. A3.01 and Raji cells ($2·5 \times 10^7$ cells/ml) were preincubated with Rho-PE-labelled DOTMA liposomes for 1 h at 37 °C in RPMI medium without phenol red, in the presence of 10% FBS or in the absence of serum. The total volume of the incubation was 1·0 ml. Free DOTMA liposomes were separated from the cells by centrifugation at 200 *g* for 10 min; DOTMA liposomes do not precipitate or sediment at this speed. The pellet was then washed twice with RPMI medium without serum. The cell-free (supernatant) and cell-associated (pellet) Rho-PE fluorescence was measured in the presence and absence of 0·5% (v/v) $C_{12}E_8$.

HIV infection of cells. Cell suspension (0·2 ml of 2×10^7 cells/ml) was incubated with HIV-1 for 2 h at 37 °C to permit adsorption of virus particles and washed three times to remove unbound virus. Cells were then resuspended at 5×10^5 cells/ml in RPMI 1640/10% FBS, cultured at 37 °C in six-well culture cluster dishes or in 25 cm^2 culture flasks, and maintained in 8 ml of medium. Mock-infected cells were treated similarly but not exposed to the virus. Samples (1 ml) of the supernatant were collected every 3 or 4 days for subsequent screening by ELISA for HIV p24 antigen content. At the same time all cell cultures were re-fed by removing two-thirds of the supernatant and replacing it with fresh medium. The effect of DOTMA liposomes on HIV infectivity was investigated using two different protocols: (i) liposomes were added to the culture medium during the exposure of cells to virus or (ii) the cells were pretreated with liposomes before addition of virus.

A3.01 cells (2×10^7 cells/ml) were preincubated with various concentrations of DOTMA liposomes for 1 h at 37 °C, washed three times to remove unbound DOTMA and then exposed to virus. After virus adsorption for 2 h at 37 °C, the cells were washed three times, resuspended at 5×10^5 cells/ml and cultured in 25 cm^2 flasks. Following incubation, cell viability was assessed visually both with and without the use of trypan blue, and p24 antigen was measured in duplicate in the supernatant. For experiments performed in the absence of serum, A3.01 cells were washed three times by centrifugation (2000 *g*, 10 min) with RPMI medium without FBS, and then resuspended and exposed to virus in the same medium.

Detection of HIV infection. HIV-specific antigens were detected in cell culture supernatants using an HIV-1 p24 antigen capture assay as described previously (Konopka *et al.*, 1990). The sensitivity of detection was routinely 38 pg of p24/ml of cell culture supernatant.

Quantification of HIV-1 binding to A3.01 cells. A3.01 cells were incubated for 2 h at 0 °C or 37 °C with HIV-1 at 10 ng of viral p24/10^6 cells in RPMI medium, in the presence of either 10% or 2% FBS. The lowest serum concentration we could achieve, while maintaining the same virus concentration, was 2%, because to obtain a concentration of 10 ng of viral p24/10^6 cells, the A3.01 cell supernatant used as a source of virus (1·0 μg p24/ml) had to be diluted fivefold. Therefore the maximum dilution of serum obtainable without changing the virus concentration was 2%. Subsequently, the cells were washed three times with medium with 10% or 2% serum and lysed with 0·5% NP40. Samples were centrifuged at 13000 *g* for 10 min and p24 antigen was measured in the supernatant. Where indicated, DOTMA liposomes, DEAE–dextran, or polybrene were added during incubation of A3.01 cells with virus.

Assay for CD4-dependent HIV-1-induced cell fusion. The assay was performed as described (Lifson, 1991) and scored semiquantitatively 24 h later. Uninfected A3.01 cells were used as indicator cells and chronically infected cells (H9/HTLV-III_B) expressing HIV glycoproteins served as effector cells; both A3.01 and H9/HTLV-III_B cells were diluted to 2×10^6 cells/ml in RPMI/10% FBS. A3.01 cells were plated in flat-bottom 96-well microtitre plates at 5×10^4 cells in 25 μl per well. Appropriate dilutions of test compounds in 200 μl of PBS were added to the wells and A3.01 cells were preincubated with the compounds for 1 h at 37 °C. Subsequently, a total of 5×10^4 H9/HTLV-III_B cells in 25 μl were added to each well containing A3.01 cells and test compound. Cells were then co-cultivated in the continuous presence of test compounds at 37 °C for 24 h. The assay was evaluated by inverted phase contrast microscopy after addition of trypan blue to wells prior to microscopic evaluation. After 24 h of co-cultivation, syncytium formation had proceeded to its full extent and cell fusion was typically extensive enough to warrant a 3+ score. A score of 4+ was assigned to numerous large syncytia with a low number of viable cells, and >4+ to numerous syncytia and no viable cells. PBS alone served as a neutral control; anti-Leu3a antibody and sCD4, known to block HIV-induced syncytium formation, served as positive controls for inhibition of cell fusion.

Detection of HIV DNA sequences by the polymerase chain reaction (PCR). K562 and Raji cells (either uninfected, or exposed to HIV-1 or heat-inactivated HIV-1 at 10 ng viral p24/10^6 cells for 2 h at 37 °C in the presence or absence of 20 μM-DOTMA liposomes) were maintained as described above. Immediately after exposure to virus, and on days 3 and 10 post-infection, K562 and Raji cells at a density of 5×10^6/ml were treated for 1 h at 56 °C in amplification buffer (50 mM-KCl, 10 mM-Tris–HCl pH 8·3, 2·5 mM-$MgCl_2$, 0·1 mg/ml gelatin) containing 0·45% NP40, 0·45% Tween 20 and 60 μg/ml proteinase K (proteinase K was subsequently inactivated at 100 °C for 10 min). Aliquots of lysate containing 10^5 cell equivalents were amplified for 35 cycles using HIV primers SK68/69 (*env*), SK38/39 (*gag*) and GH26/27 (*HLA-DQA* control), essentially according to Ou *et al.* (1988). Amplified products were hybridized to ^{32}P-labelled ATP end-labelled probes SK70, SK19 and GH64, for the *env*, *gag* and HLA primers, respectively (Ou *et al.*, 1988), and analysed on 10% polyacrylamide gels. Autoradiograms were obtained at various intervals. The reactions were performed together with negative DNA samples to control for carryover, and with a dilution series of HIV-infected cells (8E5 cells, 1 HIV proviral DNA copy/cell) (Folks *et al.*, 1986) to enable quantification of the HIV DNA sequences present in a given sample. Heat-inactivated virus controls were prepared by incubation for 1 h at 56 °C (Zack *et al.*, 1990).

Blocking experiments. Anti-Leu3a antibody has been shown to block HIV infection of T cells (Sattentau *et al.*, 1986). A3.01 cells (2×10^7 cells/ml) were washed with medium without serum, preincubated with the anti-CD4 antibody Leu3a (Becton-Dickinson) at a concentration of 10 μg/ml for 1 h at 37 °C (Weber *et al.*, 1989) and then preincubated with 10 μM-DOTMA liposomes in the absence of serum for 1 h at 37 °C. The cells were washed and infected at 1 ng of viral p24/10^6 cells in the absence of serum ($\leq$ 0·2%) or in the presence of 10% FBS. These samples were cultured for a further 10 days in the presence of 10% FBS.

RsCD4 has been shown to bind to gp120 with an affinity similar to that of membrane-bound CD4 and to inhibit HIV infection *in vitro* (Smith *et al.*, 1987; Byrn *et al.*, 1989). HIV-1 (0·4 μg viral p24/ml) was

Table 1. *Effect of the composition of the medium on fusion of HIV-1 with DOTMA liposomes*

Reaction mixture	Serum content	Initial rate*	Extent†
NaCl/TES	None	40·7	30·2
NaCl/TES	10% FBS	7·7	9·2
NaCl/TES	4 mg/ml BSA	20·2	27·0
RPMI	None	22·2	16·9
RPMI	1% FBS	14·9	20·0
RPMI	10% FBS	12·8	12·6

* Initial fusion rates were calculated from the slopes of curves from a plot of fluorescence dequenching against time. Values are given as the percentage of maximal fluorescence (F_{max})/min. Reaction medium conditions were set prior to injection of DOTMA liposomes (50 μM-lipid); virus concentration was 0·75 μg protein/ml.

† Measured as the percentage of maximal fluorescence 2·5 min after initiation of the reaction.

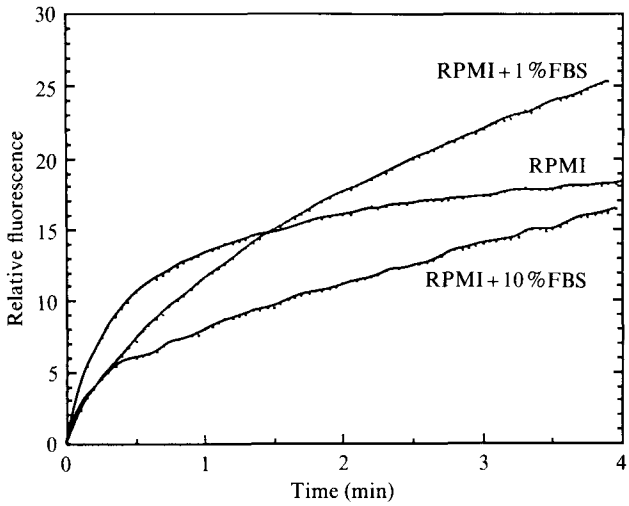

Fig. 1. Effect of the composition of the medium on fusion between HIV-1 and DOTMA liposomes. Fusion between R_{18}-labelled HIV-1 (0·75 μg/ml) and DOTMA liposomes (50 μM-lipid) (which corresponds to an approximate 133:1 liposome:virus lipid ratio) was monitored continuously at pH 7·5 as the increase in R_{18} fluorescence due to lipid mixing.

preincubated with rsCD4 (10 μg/ml) for 1 h at 37 °C, and then diluted with a cell suspension to give 1 ng of viral p24 protein/10^6 cells. A3.01 cells were infected both with virus preincubated with rsCD4 and untreated virus in the absence and presence of 2·5 μM-DOTMA liposomes. The final rsCD4 concentration during exposure of cells to preincubated virus was 0·5 μg/ml.

Results

Fusion of HIV-1 with DOTMA liposomes is dependent on the composition of the medium

We have demonstrated previously that HIV-1 fuses with DOTMA/cholesterol liposomes (Konopka *et al.*, 1990). Fusion of HIV-1 with DOTMA liposomes was dependent on the reaction conditions (Table 1), being faster in NaCl/TES than in RPMI in the absence of serum; the pH of each solution was 7·5. Addition of serum to NaCl/TES significantly reduced HIV-1 fusion with DOTMA liposomes; this effect was not caused primarily by the bovine serum albumin (BSA) content of serum (4 mg/ml is the approximate BSA concentration in 10% FBS). Addition of serum to RPMI medium had a complex effect on HIV-1 fusion with DOTMA liposomes (Table 1; Fig. 1). Although the initial rate of fusion in RPMI was reduced by roughly one-third in the presence of 1% or 10% FBS, serum had less effect on the extent of lipid mixing measured after 2·5 min. In the absence of serum, after an initial rapid increase in fluorescence intensity (representing fusion), the fluorescence quickly reached a plateau level. Addition of 10% FBS reduced the initial rate of fusion; however, the fluorescence intensity continued to increase without reaching a plateau, and after 4 min resulted in membrane fusion to an extent similar to that obtained without serum (Fig. 1). Lipid exchange was not the cause of this observed fluorescence change; there was only a slight dequenching, less than 0·5% per min, in the absence of target membrane (data not shown). HIV-1 is therefore capable of fusing with DOTMA liposomes in the presence of 10% FBS.

DOTMA liposomes can bind to but do not fuse with A3.01 and Raji cells in the presence of 10% FBS

We examined whether the presence of serum would influence the binding of Rho-PE-labelled DOTMA liposomes to $CD4^+$ A3.01 cells and $CD4^-$ Raji cells. The results were essentially comparable for both cell types in the absence of serum (Table 2). Based on the percentage of the total fluorescence that was cell-associated, approximately 1·4, 2·5 and 4·3 nmol of DOTMA liposomes were bound, in the absence of serum, to $2·5 \times 10^7$ A3.01 or Raji cells incubated with 5, 10 and 20 μM-DOTMA liposomes, respectively. The addition of 10% FBS exerted little effect on binding of DOTMA liposomes to A3.01 cells. In contrast, the association between DOTMA liposomes and Raji cells was significantly decreased in the presence of 10% FBS. The DOTMA–cell association resulted in a limited amount of lipid mixing (5 to 10%), interpreted as membrane fusion, within 1 h of incubation with both cell types in the absence of serum (data not shown). The addition of 10% FBS to the reaction mixture completely inhibited this fusion (lipid mixing).

Table 2. *Effect of serum on binding of DOTMA liposomes to A3.01 and Raji cells*

	Cell-associated liposomes (percentage of total fluorescence)*			
	A3.01 cells		Raji cells	
DOTMA (μM)	−FBS	+10% FBS	−FBS	+10% FBS
5	34·0	27·0	28·6	8·0
10	28·0	24·5	26·0	11·0
20	21·5	21·5	21·0	13·0

* A3.01 and Raji cells were preincubated with Rho-PE-labelled DOTMA liposomes as described in Methods. The percentage of the cell-associated Rho-PE was calculated as the ratio of $[F_d(c)/F_d(t)] \times 100$, where $F_d(c)$ is the cell-associated fluorescence and $F_d(t)$ is the total (cell-free plus cell-associated) fluorescence in the presence of detergent.

Table 3. *Effect of DOTMA liposomes, polybrene and DEAE-dextran on HIV-1 infection of A3.01 cells*

	p24 (ng/ml)*	
Compound added†	7 days	10 days
HIV (control)	0·3 ± 0·05	9·5 ± 2·5
5 μM-DOTMA (3·3 μg/ml)	10·8 ± 0·83	433 ± 60
30 μM-DOTMA (20 μg/ml)	35·5 ± 4·8	2458 ± 430
Polybrene (2 μg/ml)	7·9 ± 1·57	659 ± 69
Polybrene (16 μg/ml)	12·8 ± 2·4	1946 ± 398
DEAE-dextran (3 μg/ml)	2·2 ± 0·16	626 ± 119
DEAE-dextran (25 μg/ml)	40·0 ± 7·0	2055 ± 151

* p24 Antigen was determined in cell culture supernatant 7 and 10 days post-infection. Data represent mean values ± S.D. ($n = 4$).

† DOTMA liposomes (μM-lipid), polybrene and DEAE-dextran were added during exposure of A3.01 cells to HIV-1 (1 ng of viral p24/10^6 cells), as described in Methods.

Effect of DOTMA liposomes, polybrene and DEAE-dextran on HIV-1 infection of A3.01 cells

To compare the stimulatory effect of polycationic reagents on HIV-1 infectivity with that of DOTMA liposomes, A3.01 cells were infected with HIV-1 in the presence of polybrene, DEAE-dextran or DOTMA liposomes. As shown in Table 3, all three compounds, when added during exposure of A3.01 cells to virus, enhanced virus infectivity to essentially similar extents.

Enhancement of syncytium formation by DOTMA liposomes, DEAE-dextran and polybrene

Addition of polybrene, DEAE-dextran, or DOTMA liposomes during the co-cultivation of A3.01 cells with H9/HTLV-III_B cells strongly enhanced syncytium formation (Table 4). The anti-CD4 antibody Leu3a, which prevents the binding of HIV to CD4 receptors on cells (Sattentau *et al.*, 1986), and rsCD4 completely inhibited syncytium formation.

Table 4. *Enhancement of HIV-1 induced cell fusion by DOTMA liposomes, polybrene and DEAE-dextran**

Compound	Concentration	Syncytium formation
None		3+
DOTMA	2·5 to 5 μM	4+
DOTMA	10 to 30 μM	>4+
Polybrene	2 to 4 μg/ml	4+
Polybrene	8 to 32 μg/ml	>4+
DEAE-dextran	3 to 5 μg/ml	4+
DEAE-dextran	10 to 25 μg/ml	>4+
Anti-Leu3a	10 μg/ml	−
RsCD4	2·5 μg/ml	−

* Syncytia were scored 24 h after the addition of chronically infected cells H9/HTLV-III_B to uninfected A3.01 cells. The compounds were preincubated with A3.01 cells and remained in the assays during the co-incubation period.

Table 5. *Effect of RsCD4 on HIV-1 infection of A3.01 cells, in the presence or absence of DOTMA liposomes*

Time post-infection (days)	Treatment			
	HIV (sCD4)* + DOTMA	HIV (sCD4)*	HIV + DOTMA	HIV
7	1·1 ± 0·14†	0·18 ± 0·05	58·5 ± 15·6	2·8 ± 0·26
10	27·2 ± 0·62	4·22 ± 0·86	1750 ± 17·3	93·0 ± 6·2

* HIV-1 was preincubated with sCD4 (10 μg/ml) for 1 h at 37 °C. A3.01 cells were infected with untreated virus and the sCD4-preincubated HIV (1 ng of viral p24/10^6 cells), in the absence of DOTMA or in the presence of 2·5 μM-DOTMA liposomes as described in Methods.

† p24 Antigen was determined in cell culture supernatant 7 and 10 days post-infection. Data represent mean p24 ng/ml ± S.D. ($n = 4$).

RsCD4 inhibits the enhancement of HIV-1 infectivity by DOTMA liposomes in A3.01 cells

Anti-Leu3a antibody blocked 93% of the infectivity of HIV-1 in the presence of DOTMA liposomes (Konopka *et al.*, 1990). To investigate further the role of CD4 in the DOTMA-mediated enhancement of HIV-1 infectivity in A3.01 cells, we used rsCD4 to inhibit infection in the absence and presence of DOTMA liposomes (Table 5). Preincubation of HIV-1 with rsCD4 (10 μg/ml) resulted in inhibition of virus infectivity by 94%. When A3.01

Table 6. *Effect of serum on the DOTMA-mediated enhancement of HIV-1 infectivity in A3.01 cells*

Viral p24 (ng/10^6 cells)	FBS (%)	−DOTMA	+DOTMA*
1	0·2†	19·9 ± 1·7‡	18·7 ± 1·8
1	10	3·4 ± 0·1	42·6 ± 8·5
2	0·4	59·2 ± 4·7	39·4 ± 2·3
2	10	6·1 ± 0·5	136·5 ± 23·8
4	0·8	81·0 ± 5·6	159·2 ± 9·3
4	10	11·9 ± 0·7	374·5 ± 88·1
5	1·0	131·1 ± 13·9	199·0 ± 13·3
5	10	10·8 ± 1·6	375·5 ± 15·8
10	2·0	187·3 ± 24·5	482·5 ± 82·0
10	10	29·4 ± 2·4	1268·0 ± 95·7

* 10 μM-DOTMA liposomes was added during exposure of A3.01 cells to HIV-1 in the presence of the indicated concentration of FBS, as described in Methods.

† A3.01 cells washed with medium without serum were infected as described in Methods.

‡ p24 Antigen was determined in cell culture supernatant 7 days post-infection. Data represent mean p24 ng/ml ± S.D. ($n = 4$).

Table 7. *Effect of preincubation of A3.01 cells with DOTMA liposomes on HIV-1 infectivity*

	7 days	10 days
Preincubation − FBS*		
+ HIV (control)	2·4 ± 0·24†	58·3 ± 1·7
1·0 μM-DOTMA	2·3 ± 0·63	112·8 ± 2·5
2·5 μM-DOTMA	4·7 ± 0·62	224·0 ± 27·6
10·0 μM-DOTMA	16·1 ± 1·8	319·5 ± 30·0
20·0 μM-DOTMA	68·0 ± 10·8	1248·8 ± 129
Preincubation + 10% FBS*		
+ HIV (control)	0·9 ± 0·03	30·3 ± 1·3
1·0 μM-DOTMA	4·0 ± 0·43	95·0 ± 12·8
2·5 μM-DOTMA	7·5 ± 0·7	361·0 ± 35·4
10·0 μM-DOTMA	29·0 ± 3·9	605·0 ± 48
20·0 μM-DOTMA	77·3 ± 11·9	1075·0 ± 151·5

* A3.01 cells were preincubated with DOTMA liposomes, washed and exposed to HIV-1 (1 ng of viral p24/10^6 cells), as described in Methods.

† p24 Antigen was determined in cell culture supernatant 7 and 10 days post-infection. Data represent mean p24 ng/ml ± S.D. ($n = 4$).

cells were infected with virus pretreated with rsCD4 in the presence of 2·5 μM-DOTMA liposomes, the amount of p24 was reduced to 1·8% of the amount produced by cells infected with untreated HIV-1 in the presence of the liposomes.

Effect of serum on the DOTMA-mediated enhancement of HIV-1 infectivity in A3.01 cells

We examined whether DOTMA liposomes would enhance infection to the same extent both in the presence of 10% FBS and when the serum content was reduced to $\leqslant 2\%$ by dilution of virus stock (Table 6). A3.01 cells were washed with medium without serum and infected at 1, 2, 4, 5 and 10 ng of viral p24/10^6 cells, corresponding to 0·2, 0·4, 0·8, 1·0 and 2·0% FBS, respectively, in the absence of liposomes or in the presence of 10 μM-DOTMA liposomes. These samples were further cultured for 7 days in the continuous presence of 10% FBS as described in Methods. In a parallel experiment, cells were infected in medium with 10% FBS.

In the absence of DOTMA liposomes, HIV-1 infection of A3.01 cells was inhibited significantly by 10% FBS (Table 6). DOTMA had little or no effect on HIV-1 infection at low FBS concentrations (0·2 and 0·4%). Approximately twofold enhancement of HIV-1 infectivity was observed in the presence of 0·8 to 2·0% FBS when compared to A3.01 cells infected in the absence of DOTMA. The DOTMA-mediated enhancement was significantly higher when cells were exposed to virus in the presence of 10% FBS; HIV-1 infection was increased by 12·5- to 43-fold, depending on the virus inoculum, compared to cells exposed to virus in the absence of DOTMA.

Preincubation of A3.01 cells with DOTMA liposomes enhances HIV-1 infectivity

To determine whether pretreatment of A3.01 cells with DOTMA liposomes could affect HIV-1 infectivity, the cells were preincubated with various concentrations of DOTMA liposomes (1 to 20 μM-lipid), both in the presence of 10% FBS and without serum. The cells were then washed and exposed to virus, as described in Methods, and cultured for 10 days (Table 7). Pretreatment of A3.01 cells with DOTMA liposomes resulted in concentration-dependent enhancement of HIV-1 infectivity. The DOTMA-mediated enhancement of HIV-1 infection, as determined by p24 production, was not affected by the presence of 10% FBS during pretreatment of A3.01 cells with liposomes. As discussed above, essentially no fusion (lipid mixing) was observed between A3.01 cells and DOTMA liposomes under these conditions (data not shown), although binding did take place (Table 2).

DOTMA liposomes, DEAE–dextran and polybrene enhance HIV-1 binding to A3.01 cells

To test the influence of the polycationic compounds on the interaction between cells and HIV-1, A3.01 cells were incubated with virus in the presence of 2% and 10% FBS, at 0 °C and 37 °C, washed, lysed and then assayed

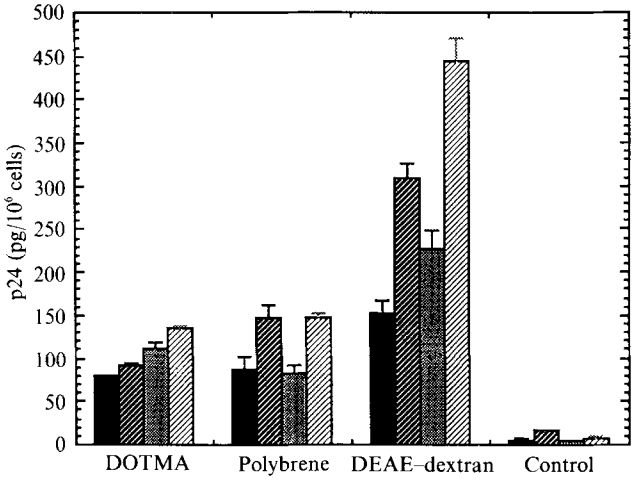

Fig. 2. Effect of DOTMA liposomes (10 μM-lipid), polybrene (16 μg/ml) and DEAE–dextran (25 μg/ml) on HIV-1 binding to A3.01 cells. Cells were exposed to HIV-1 (10 ng of viral p24/10^6 cells), in the presence of 2% or 10% FBS, for 2 h at 0 °C (■, with 2% FBS; ▩, with 10% FBS) or 37 °C (▨, with 2% FBS; ▨, with 10% FBS), and then washed and lysed as described in Methods. Data represent mean values ± S.D. ($n = 4$). The control values are too small to be shown at this scale and are given in the text.

for p24 antigen to quantify HIV-1 binding to the cells (Fig. 2). Co-incubation of the virus with any one of the three polycationic compounds enhanced the amount of virus bound to A3.01 cells substantially, as determined by the amount of p24 antigen in the cell lysate, following washing. The amount of p24 antigen determined after incubation at 37 °C was greater than at 0 °C. The amount of p24 determined in the lysate of control cells, with 2% FBS present during incubation, was 5·8 ± 0·4 and 16·5 ± 3·2 pg/10^6 cells, at 0 °C and 37 °C respectively. The greater amounts of p24 obtained at 37 °C may reflect both the increased binding between gp120 and the CD4 receptor, and virus entry (penetration) into the target cells. DOTMA liposomes and DEAE–dextran were more effective when incubation of A3.01 cells with HIV-1 was performed in the presence of 10% FBS, whereas in the case of polybrene, binding was unaffected by an increase in FBS concentration from 2 to 10%. Furthermore, 10% FBS inhibited the binding of HIV-1 to A3.01 cells in the absence of polycationic compounds. The amount of p24 antigen determined in the lysate of control cells, with 10% FBS being present during incubation, was 3·8 ± 1·1 and 7·9 ± 1·3 pg/10^6 cells at 0 °C and 37 °C respectively. Thus, the increase in the concentration of FBS from 2 to 10% inhibited the binding of HIV-1 to A3.01 cells by 35% and 52% at 0 °C and 37 °C, respectively. Since the inhibition of HIV-1 binding by FBS also causes inhibition of infectivity (measured by p24 production after 7 days; unpublished data), and because infection of A3.01 cells is CD4-dependent, it

Table 8. *Effect of preincubation of A3.01 cells with anti-Leu3a antibodies and DOTMA liposomes on HIV-1 infectivity*

Treatment			
+ DOTMA ⩽ 0·2% FBS	+ DOTMA 10% FBS	− DOTMA ⩽ 0·2% FBS	− DOTMA 10% FBS
A3.01 cells + anti-Leu3a antibody*			
1·6 ± 0·13†	4·5 ± 0·36	0·36 ± 0·05	0·3 ± 0·05
A3.01 cells − anti-Leu3a antibody			
39·8 ± 5·7	75·3 ± 2·5	26·2 ± 2·5	4·7 ± 0·2

* A3.01 cells were preincubated with anti-Leu3a antibody (10 μg/ml) for 1 h at 37 °C and then preincubated in the absence or presence of 10 μM-DOTMA liposomes in the absence of serum, as described in Methods. The cells were washed and infected at 1 ng of viral p24/10^6 cells in the absence of serum (⩽ 0·2% FBS) or in the presence of 10% FBS.

† p24 Antigen was determined in cell culture supernatant 10 days post-infection. Data represent mean p24 ng/ml ± S.D. ($n = 4$).

appears that some component of binding inhibition is CD4-specific. However, we cannot exclude the possibility that part of the inhibition is due to a reduction of non-specific binding. Less effective binding of HIV-1 to the cells at the higher serum concentration may be responsible for the reduced infection observed in the presence of 10% FBS (Table 6). These data suggest that adsorption of HIV-1 to the surface of $CD4^+$ A3.01 cells was strongly dependent on the composition of the medium and that the enhancing action of polycationic reagents was largely at the adsorption step.

Anti-Leu3a antibody inhibits the DOTMA-mediated enhancement of HIV-1 infectivity

Our previous experiments on the effect of anti-Leu3a antibody on HIV-1 infectivity (Konopka *et al.*, 1990) were performed in the presence of 10% FBS when no fusion of DOTMA liposomes with cell membranes occurred. The question arises whether fusion of DOTMA liposomes with cell membranes could enhance HIV-1 infection independently of CD4. If this is the case, then preincubation of anti-Leu3a-pretreated cells with DOTMA liposomes, under conditions in which limited lipid mixing occurred (i.e. in the absence of serum), should enhance the infectivity of HIV-1, compared to infection of non-DOTMA-pretreated cells.

A3.01 cells were preincubated with anti-Leu3a antibody, and then with DOTMA liposomes in the absence of serum, as described in Methods. Preincubation of A3.01 cells with anti-Leu3a alone reduced the infectivity of HIV-1 by approximately 95% 10 days post-infection, when infection was performed either in the presence of 10% FBS or in the absence of serum (⩽0·2%) (Table 8).

When anti-Leu3a- and DOTMA-pretreated cells were exposed to HIV-1 in the presence of 10% FBS or in the absence or serum ($\leqslant 0{\cdot}2\%$), the amount of p24 was also reduced to about 5% of the that produced by cells pretreated with liposomes, but without pretreatment with antibody. Thus, anti-Leu3a antibody inhibited p24 production to a similar extent in DOTMA-pretreated and untreated cells, suggesting that blocking of the CD4 receptor with anti-Leu3a antibody greatly diminished the DOTMA-mediated enhancement of infectivity.

Effect of DOTMA liposomes on HIV infection of K562 and Raji cells

We investigated whether the $CD4^-$ K562 and Raji cells could be infected with HIV-1 in the presence of DOTMA liposomes. Raji cells were chosen because previous studies had indicated that if these cells are transfected so that they express CD4 on their surface, they are susceptible to HIV-1 infection (Maddon *et al.*, 1986). These cells were not susceptible to HIV-1 infection (10 ng viral p24 added/10^6 cells), both in the absence or presence of 20 μM-DOTMA liposomes. No p24 could be detected in the cell culture supernatant 3 and 10 days post-infection (data not shown). Immediately after exposure to virus, a very low amount of HIV proviral DNA (between 2 and 10 copies/10^5 cells) was detected by PCR amplification both in K562 and Raji cells, infected in the absence or presence of 20 μM-DOTMA liposomes. Essentially the same amount of proviral DNA was detected in cells treated with heat-inactivated virus, although the amount of HIV-1 DNA detected in the cells after 3 days of culture was significantly lower and was essentially undetectable on day 10 post-infection (data not shown). The results suggest that a low amount of viral DNA represents contaminating viral DNA from the virus added to the exposed cells, indicating that the presence of DOTMA liposomes did not cause HIV-1 infection and replication in $CD4^-$ K562 and Raji cells.

Discussion

Our previous studies have shown that preincubation of HIV-1 with DOTMA liposomes or the presence of DOTMA liposomes during infection enhances the infectivity of HIV-1 (Konopka *et al.*, 1990). The positive charge of DOTMA is likely to be important in increased virus infectivity, because the addition of polyanions such as DNA (Konopka *et al.*, 1990) or oligodeoxynucleotides (unpublished results) can abolish DOTMA-mediated enhancement. Similar neutralization has been reported for polycation-mediated enhancement of virus adsorption for avian sarcoma viruses (Toyoshima & Vogt, 1969). DOTMA-mediated enhancement has been observed even in the presence of 10% FBS, which inhibits the fusion (lipid mixing) of DOTMA-containing liposomes with cell membranes and subsequent transfection (Felgner *et al.*, 1987; this study). Although the initial rate of fusion between HIV-1 and DOTMA liposomes was reduced after the addition of serum to RPMI, the time course of fusion (Fig. 1) indicates that during a 2 h incubation of HIV-1 with DOTMA liposomes in RPMI medium containing 10% FBS (Konopka *et al.*, 1990), virus and liposomes can fuse to a significant extent. Further studies are necessary to quantify the fusion of DOTMA liposomes with HIV in the presence of human serum.

In contrast, DOTMA liposomes did not fuse with A3.01 or Raji cells in serum-containing medium, but fused to a limited extent only in the absence of serum. However, HIV-1 infectivity was enhanced when A3.01 cells were preincubated with DOTMA liposomes, both in the presence and absence of 10% FBS (Table 7). Furthermore, DOTMA-treated cells could be washed several times without losing their increased susceptibility to infection. These results suggest that the binding of DOTMA liposomes to A3.01 cells (in the absence of any fusion) is sufficient to enhance HIV-1 infectivity. Liposomes containing DOTMA and PE can fuse with other liposomes containing negatively charged phospholipids (Düzgüneş *et al.*, 1989). Therefore, it is possible that in the absence of serum, binding of DOTMA to negatively charged residues near the surface of the lipid bilayer may lead to destabilization of the plasma membrane and subsequent lipid mixing (fusion). Although fusion is inhibited in the presence of serum, DOTMA liposomes still bind to the negatively charged cell membrane. The attachment of DOTMA or DOTMA–serum complexes to the cell surface therefore appears to increase virus–cell binding. The enhancement of infectivity could arise from (i) increased binding of the virus to the cell surface, followed by the normal fusion reaction, (ii) increased fusogenicity of the DOTMA–HIV fusion product or (iii) a more efficient fusion step following binding (e.g. an accelerated conformational change in gp120 or shedding of gp120 from the virions; Moore *et al.*, 1990).

Interestingly, both HIV-1 binding to the cell surface and HIV-1 infection of A3.01 cells was significantly inhibited by 10% FBS. Deglycosylation of gp120 and gp41 significantly reduces, but does not abolish, HIV-1 binding to and infectivity of $CD4^+$ cells (Fenouillet *et al.*, 1990). Human serum mannose-binding lectins are able to bind to gp120 and this may lead to blockage of infection *in vitro* (Lifson *et al.*, 1986*b*; Robinson *et al.*, 1987; Ezekowitz *et al.*, 1989). On the other hand, Larkin *et al.* (1989) have reported that lectins do not inhibit HIV-1-

mediated syncytium formation between HIV-1_{RF}-infected H9 cells and C8166 (T cell leukaemia) indicator cells. Sera from normal, HIV-uninfected subjects reduce the sensitivity of an ELISA for gp120 with rsCD4 in the solid phase, suggesting that serum components interfere with gp120–CD4 binding (Gilbert *et al.*, 1991). In a focal infectivity assay which measures the production of distinct foci of HIV infection in a monolayer of $CD4^+$ HeLa cells (Pincus *et al.*, 1991), plaque formation by supernatant HIV is inhibited by 10% FBS. Binding between gp120 and mannose- and carbohydrate-binding proteins present in FBS (Kawasaki *et al.*, 1985) may be responsible for the inhibitory effects of serum observed in our studies. DOTMA liposomes appear to overcome the inhibitory effect of FBS by enhancing the binding between HIV-1 and $CD4^+$ cells. Alternatively, DOTMA may bind to an inhibitory factor in FBS and neutralize its effect. Arguing against this possibility, however, is the observation that DOTMA-mediated enhancement of infectivity is higher in the presence of 10% FBS than at lower serum concentrations (Table 6); more of this factor would be removed by DOTMA at lower FBS concentrations. It is unclear why the DOTMA-mediated enhancement is significantly higher in the presence of 10% FBS than at lower concentrations.

Polybrene or DEAE–dextran have been used to increase the efficiency of HIV infection *in vitro* in both $CD4^-$ and $CD4^+$ cells with varying effectiveness depending on the HIV isolate (Cheng-Mayer *et al.*, 1987; Chesebro & Wehrly, 1988; Zaia *et al.*, 1988; Tateno *et al.*, 1989; Busso & Resnick, 1990; Ikeuchi *et al.*, 1990). Chesebro & Wehrly (1988) have reported that the infection of HeLa $CD4^+$ cells by HIV (LAV) is partially inhibited by DEAE–dextran and strongly inhibited by polybrene. Primary HIV isolates could infect HeLa $CD4^+$ cells only when pretreated with DEAE–dextran; incubation without pretreatment with DEAE–dextran or in the presence of polybrene did not result in infection. Polybrene also inhibits the focal infectivity assay with HeLa $CD4^+$ cells (Pincus *et al.*, 1991). Ikeuchi *et al.* (1990) have shown that several $CD4^-$ mesenchymal cells of different origin, including foreskin fibroblasts (F13), chondrocytes (C23) and synovial cells (HSA), can be infected with HIV-1 or -2. All the cell types could be infected by HIV-2 whether or not DEAE–dextran was present, whereas DEAE–dextran was required for HIV-1 infection of C23 or HSA cells; F13 cells could not be infected by HIV-1 under any conditions. In our studies, DOTMA liposomes, DEAE–dextran and polybrene enhanced HIV-1 infection of A3.01 cells, and syncytium formation between H9/HTLV-III_B and A3.01 cells to an essentially similar extent, although DEAE–dextran increased the amount of HIV-1 bound to A3.01 more effectively than DOTMA or polybrene. DEAE–dextran and DOTMA liposomes were more effective when the concentration of FBS was increased from 2% to 10%, whereas in the case of polybrene, binding was unaffected by the increase. These studies illustrate the complexity of the effect of polycationic agents on HIV infectivity.

Our observations contrast with the results of Innes *et al.* (1990) who reported that lipofectin (a commercial preparation containing DOTMA and dioleoyl PE) mediates the infection of non-permissive cells by murine leukaemia virus, but does not enhance the infection of permissive cells. Lipofectin-mediated infection of normally non-permissive cells is inhibited by FBS, but the infection of permissive cells in the presence of lipofectin is slightly enhanced. Innes *et al.* (1990) found that polybrene alone stimulates the infection of permissive cells, but is unable to mediate the infection of non-permissive cells. It seems likely that the effect of polycationic reagents and serum on the infectivity of retroviruses is strongly dependent on the mechanisms involved in (i) the attachment of the virus to the cell membrane and (ii) the penetration of virus into the host cell.

Lipofectin has been used as an efficient reagent for the introduction of nucleic acids into cells (Felgner *et al.*, 1987; Malone *et al.*, 1989). The observation that DOTMA liposomes enhance virus infectivity raises the following possibility: in experiments where HIV or simian immunodeficiency virus DNA is transfected using lipofectin, intact virions produced by the host cells may re-infect cells by means of residual surface-bound lipofectin after the transfection process. A similar argument may hold for DEAE–dextran.

Our studies have confirmed our previous observations (Konopka *et al.*, 1990) that the enhancement of infectivity by DOTMA liposomes takes place primarily via the CD4 receptor; DOTMA liposomes do not mediate HIV-1 infection and replication in $CD4^-$ cell lines. In the presence of DOTMA liposomes, rsCD4 and anti-Leu3a antibody inhibit the infectivity of HIV-1 by 98% and 94%, respectively. Blocking infection with rsCD4 (Smith *et al.*, 1987; Byrn *et al.*, 1989) or anti-Leu3a antibody (Sattentau *et al.*, 1986; Weber *et al.*, 1989) has been taken as an indication that HIV-1 entry occurs predominantly via the CD4 receptor. DOTMA liposomes enhance the infectivity of the HTLV-III_B strain in H9 cells, but are unable to enhance infection in chronically infected, CD4-downregulated, H9/HTLV-III_B cells, as measured by p24 production during propagation (unpublished data).

It has been shown by Maddon *et al.* (1986) that CD4 expressed in HeLa cells can function as a receptor for HIV and render these cells susceptible to HIV infection. In contrast, HIV can bind to murine cells (e.g. NIH3T3) transfected to express CD4, but productive infection is

not observed. This difference in infectivity exists even though the affinity of Leu3a for CD4 expressed on transfected murine and human cells is very similar, the number of CD4 molecules expressed is comparable and the endocytic pathway of CD4 is the same (Pelchen-Matthews *et al.*, 1989). It will be of interest to determine whether NIH3T3 cells expressing transfected CD4 can be infected with HIV in the presence of DOTMA liposomes, and compare the results with those obtained with HeLa cells expressing CD4. These studies are in progress in our laboratory.

Biochemical studies of viral proteins may benefit from increased virus production, as demonstrated by the use of DOTMA liposomes in the initial stage of infection. This technique has already been applied to the study of glycosylation of gp120 produced in U937 and A3.01 cells (C. A. Abel, E. Pretzer, M. White, H. Enkel & B. Davis. Abstracts of the Universitywide AIDS Research Program Annual Meeting, San Diego, 1990 and San Francisco, 1991). Another benefit of DOTMA-mediated enhancement of HIV-1 infectivity may be the increased propagation of natural HIV isolates as well as laboratory strains.

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References

ALOIA, R. C., JENSEN, F. C., CURTAIN, C. C., MOBLEY, O. W. & GORDON, L. M. (1988). Lipid composition and fluidity of the human immunodeficiency virus. *Proceedings of the National Academy of Sciences, U.S.A.* **85**, 900–904.

BANGHAM, A. D., STANDISH, M. M. & WATKINS, J. C. (1965). Diffusion of univalent ions across lamellae of swollen phospholipids. *Journal of Molecular Biology* **13**, 238–252.

BOSCH, M. L., EARL, P. L., FARGNOLI, K., PICCIAFUOCO, S., GIOMBINI, F., WONG-STAAL, F. & FRANCHINI, G. (1989). Identification of the fusion peptide of primate immunodeficiency viruses. *Science* **244**, 694–697.

BUSSO, M. E. & RESNICK, L. (1990). Anti-human immunodeficiency virus effects of dextran sulfate are strain dependent and synergistic or antagonistic when dextran sulfate is given in combination with dideoxynucleosides. *Antimicrobial Agents and Chemotherapy* **34**, 1991–1995.

BYRN, R. A., SEKIGAVA, I., CHAMOW, S. M., JOHNSON, J. S., GREGORY, T. J., CAPON, D. J. & GROOPMAN, J. E. (1989). Characterization of *in vitro* inhibition of human immunodeficiency virus by purified recombinant CD4. *Journal of Virology* **63**, 4370–4375.

CAO, Y., FRIEDMAN-KIEN, A. E., HUANG, Y., LI, X. L., MIRABILE, M., MOUDGIL, T., ZUCKER-FRANKLIN, D. & HO, D. D. (1990). CD4-independent, productive human immunodeficiency virus type 1 infection of hepatoma cell lines *in vitro*. *Journal of Virology* **64**, 2553–2559.

CENTERS FOR DISEASE CONTROL (1988). 1988 Agent summary statement for human immunodeficiency virus and report on laboratory-acquired infection with human immunodeficiency virus. *Morbidity and Mortality Weekly Report* **37** (Supplement S-4), 1–17.

CHENG-MAYER, C., RUTKA, J. T., ROSENBLUM, M. L., MCHUGH, T., STITES, D. P. & LEVY, J. A. (1987). Human immunodeficiency virus can productively infect cultured human glial cells. *Proceedings of the National Academy of Sciences, U.S.A.* **84**, 3526–3530.

CHESEBRO, B. & WEHRLY, K. (1988). Development of a sensitive quantitative focal assay for human immunodeficiency virus infectivity. *Journal of Virology* **62**, 3779–3788.

CHESEBRO, B., BULLER, R., PORTIS, J. & WEHRLY, K. (1990). Failure of human immunodeficiency virus entry and infection in CD4-positive human brain and skin cells. *Journal of Virology* **64**, 215–221.

CLAPHAM, P. R., WEBER, J. N., WHITBY, D., MCINTOSH, K., DALGLEISH, A. G., MADDON, P. J., DEEN, K. C., SWEET, R. W. & WEISS, R. A. (1989). Soluble CD4 blocks the infectivity of diverse strains of HIV and SIV for T cells and monocytes but not for brain and muscle cells. *Nature, London* **337**, 368–370.

DALGLEISH, A. G., BEVERLEY, P. C. L., CLAPHAM, P. R., CRAWFORD, D. H., GREAVES, M. F. & WEISS, R. A. (1984). The CD4(T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature, London* **312**, 763–767.

DEBS, R. J., FREEDMAN, L. P., EDMUNDS, S., GAENSLER, K. L., DÜZGÜNEŞ, N. & YAMAMOTO, K. R. (1990). Regulation of gene expression *in vivo* by liposome-mediated delivery of a purified transcription factor. *Journal of Biological Chemistry* **265**, 10189–10192.

DEWHURST, S., SAKAI, K., BRESSER, J., STEVENSON, M., EVINGER-HODGES, M. J. & VOLSKY, D. J. (1987). Persistent productive infection of human glial cells by human immunodeficiency virus (HIV) and by infectious molecular clones of HIV. *Journal of Virology* **61**, 3774–3782.

DÜZGÜNEŞ, N. & BENTZ, J. (1988). Fluorescence assays for membrane fusion. In *Spectroscopic Membrane Probes*, pp. 117–159. Edited by L. Loew. Boca Raton: CRC Press.

DÜZGÜNEŞ, N., WILSCHUT, J., HONG, K., FRALEY, R., PERRY, C., FRIEND, D. S., JAMES, T. L. & PAPAHADJOPOULOS, D. (1983). Physicochemical characterization of large unilamellar vesicles prepared by reverse-phase evaporation. *Biochimica et biophysica acta* **732**, 289–299.

DÜZGÜNEŞ, N., GOLDSTEIN, J. A., FRIEND, D. S. & FELGNER, P. L. (1989). Fusion of liposomes containing a novel cationic lipid, *N*-[2,3-(dioleyloxy)propyl]-*N*,*N*,*N*-trimethylammonium: induction by multivalent anions and asymmetric fusion with acidic phospholipid vesicles. *Biochemistry* **28**, 9179–9184.

EZEKOWITZ, A. B., KUHLMAN, M., GROOPMAN, J. E. & BYRN, R. A. (1989). A human serum mannose-binding protein inhibits *in vitro* infection by the human immunodeficiency virus. *Journal of Experimental Medicine* **169**, 185–196.

FELGNER, P. L. & RINGOLD, G. M. (1989). Cationic liposome-mediated transfection. *Nature, London* **337**, 387–388.

FELGNER, P. L., GADEK, T. R., HOLM, M., ROMAN, R., CHAN, H. W., WENZ, M., NORTHROP, J. P., RINGOLD, G. M. & DANIELSEN, M. (1987). Lipofectin: a highly efficient, lipid-mediated DNA-transfection procedure. *Proceedings of the National Academy of Sciences, U.S.A.* **84**, 7413–7417.

FENOUILLET, E., GLUCKMAN, J. C. & BAHRAOUI, E. (1990). Role of *N*-linked glycans of envelope glycoproteins in infectivity of human immunodeficiency virus type 1. *Journal of Virology* **64**, 2841–2848.

FOLKS, T. M., POWELL, D., LIGHTFOOTE, M., KOENIG, S., FAUCI, A. S., BENN, S., RABSON, A., DAUGHERTY, D., GENDELMAN, H. E., HOGGAN, M. D., VENKATESAN, S. & MARTIN, M. A. (1986). Biological and biochemical characterization of a cloned Leu-3$^-$ cell surviving infection with the acquired immune deficiency syndrome retrovirus. *Journal of Experimental Medicine* **164**, 280–290.

GARTNER, S., MARKOVITZ, P., MARKOVITZ, D. M., KAPLAN, M. H., GALLO, R. C. & POPOVIC, M. (1986). The role of mononuclear phagocytes in HTLV-III/LAV infection. *Science* **233**, 215–219.

GILBERT, M., KIRIHARA, J. & MILLS, J. (1991). Enzyme-linked immunoassay for human immunodeficiency virus type 1 envelope glycoprotein 120. *Journal of Clinical Microbiology* **29**, 142–147.

HAROUSE, J. M., KUNSCH, C., HARTLE, H. T., LAUGHLIN, M. A., HOXIE, J. A., WIGDAHL, B. & GONZALES-SCARANO, F. (1989). CD4-independent infection of human neural cells by human immunodeficiency virus type 1. *Journal of Virology* **63**, 2527–2533.

HO, D. D., ROTA, T. R. & HIRSCH, M. S. (1986). Infection of monocyte/macrophages by human T lymphotropic virus type III. *Journal of Clinical Investigation* **77**, 1712–1715.

HOEKSTRA, D., DE BOER, T., KLAPPE, K. & WILSCHUT, J. (1984). Fluorescence method for measuring the kinetics of fusion between biological membranes. *Biochemistry* **23**, 5675–5681.

HOFFMAN, A. D., BANAPOUR, B. & LEVY, J. A. (1985). Characterization of the AIDS-associated retrovirus reverse transcriptase and optimal conditions for its detection in virions. *Virology* **147**, 326–335.

HOPE, M. J., BALLY, M. B., WEBB, G. & CULLIS, P. R. (1985). Production of large unilamellar vesicles by a rapid extrusion procedure. Characterization of size distribution, trapped volume and ability to maintain a membrane potential. *Biochimica et biophysica acta* **812**, 55–65.

IKEUCHI, K., KIM, S., BYRN, R. A., GOLDRING, S. R. & GROOPMAN, J. E. (1990). Infection of nonlymphoid cells by human immunodeficiency virus type 1 or type 2. *Journal of Virology* **64**, 4226–4231.

INNES, C. L., SMITH, P. B., LANGENBACH, R., TINDALL, K. R. & BOONE, L. R. (1990). Cationic liposomes (lipofectin) mediate retroviral infection in the absence of specific receptors. *Journal of Virology* **64**, 957–961.

KAWASAKI, N., KAWASAKI, T. & YAMASHINA, I. (1985). Mannan-binding protein and conglutinin in bovine serum. *Journal of Biochemistry* **98**, 1309–1320.

KLATZMANN, D., CHAMPAGNE, E., CHAMARET, S., GRUEST, J., GUETARD, D., HERCEND, T., GLUCKMAN, J.-C. & MONTAGNIER, L. (1984). T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. *Nature, London* **312**, 767–768.

KONOPKA, K., DAVIS, B. R., LARSEN, C. E., ALFORD, D. R., DEBS, R. J. & DÜZGÜNEŞ, N. (1990). Liposomes modulate human immunodeficiency virus infectivity. *Journal of General Virology* **71**, 2899–2907.

KONOPKA, K., DAVIS, B. R. & DÜZGÜNEŞ, N. (1991). HIV-1 infection of a non-CD4-expressing variant of HUT-78 cells: lack of inhibition by Leu3a antibodies and enhancement by cationic DOTMA liposomes. In *Mechanisms and Specificity of HIV Entry into Host Cells*. Edited by N. Düzgüneş. New York: Plenum Press (in press).

KOWALSKI, M., POTZ, J., BASIRIPOUR, L., DORFMAN, T., GOH, W. C., TERWILLIGER, E., DAYTON, A., ROSEN, C., HASELTINE, W. & SODROSKI, J. (1987). Functional regions of the envelope glycoprotein of human immunodeficiency virus type 1. *Science* **237**, 1351–1355.

KOWALSKI, M., BERGERON, L., DORFMAN, T., HASELTINE, W. & SODROSKI, J. (1991). Attentuation of human immunodeficiency virus type 1 cytopathic effect by a mutation affecting the transmembrane envelope glycoprotein. *Journal of Virology* **65**, 281–291.

LARKIN, M., CHILDS, R. A., MATTHEWS, T. J., THIEL, S., MIZUOCHI, T., LAWSON, A. M., SAVILL, J. S., HASLETT, C., DIAZ, R. & FEIZI, T. (1989). Oligosaccharide-mediated interactions of the envelope glycoprotein gp120 of HIV-1 that are independent of CD4 recognition. *AIDS* **3**, 793–798.

LARSEN, C. E., ALFORD, D. R., YOUNG, L. J. T., MCGRAW, T. P. & DÜZGÜNEŞ, N. (1990). Fusion of simian immunodeficiency virus with liposomes and erythrocyte ghosts: effect of liposome composition, pH and calcium. *Journal of General Virology* **71**, 1947–1955.

LASKY, L. A., NAKAMURA, G., SMITH, D. H., FENNIE, C., SHIMASAKI, C., PATZER, E., BERMAN, P., GREGORY, T. & CAPON, D. J. (1987). Delineation of a region of the human immunodeficiency virus type 1 gp120 glycoprotein critical for interaction with the CD4 receptor. *Cell* **50**, 975–985.

LI, X. L., MOUDGIL, T., VINTERS, H. V. & HO, D. D. (1990). CD4-independent productive infection of a neuronal cell line by human immunodeficiency virus type 1. *Journal of Virology* **64**, 1383–1387.

LIFSON, J. D. (1991). Fusion of HIV-infected cells with uninfected cells; CD4-dependent HIV-induced cell fusion. In *Membrane Fusion Techniques, Method in Enzymology*. Edited by N. Düzgüneş. New York: Academic Press (in press).

LIFSON, J. D., FEINBERG, M. B., REYES, G. R., RABIN, L., BANAPOUR, B., CHAKRABARTI, S., MOSS, B., WONG-STAAL, F., STEIMER, K. S. & ENGLEMAN, E. G. (1986*a*). Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. *Nature, London* **323**, 725–728.

LIFSON, J., COUTRE, S., HUANG, E. & ENGLEMAN, E. (1986*b*). Role of envelope glycoprotein carbohydrate in human immunodeficiency virus (HIV) infectivity and virus-induced cell fusion. *Journal of Experimental Medicine* **64**, 2101–2120.

LONGE, P., CABIAUX, V., LONG, L. & RUYSSCHAERT, J. M. (1986). Fusion of Newcastle disease virus with liposomes: role of the lipid composition of liposomes. *Biochimica et biophysica acta* **858**, 312–316.

LOYTER, A., CITOVSKY, V. & BLUMENTHAL, R. (1988). The use of fluorescence dequenching measurements to follow viral membrane fusion events. *Methods of Biochemical Analysis* **33**, 129–164.

MCDOUGAL, S. J., NICHOLSON, J. K. A., CROSS, G. D., CORT, S. P., KENNEDY, M. S. & MAWLE, A. C. (1986). Binding of the human retrovirus HTLV-III/LAV/ARV/HIV to the CD4 (T4) molecule: conformation dependence, epitope mapping, antibody inhibition, and potential for idiotypic mimicry. *Journal of Immunology* **137**, 2937–2944.

MADDON, P. J., DALGLEISH, A. G., MCDOUGAL, J. S., CLAPHAM, P. R., WEISS, R. A. & AXEL, R. (1986). The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47**, 333–348.

MALONE, R. W., FELGNER, P. L. & VERMA, I. M. (1989). Cationic liposome-mediated RNA transfection. *Proceedings of the National Academy of Sciences, U.S.A.* **86**, 6077–6081.

MOORE, J. P., MCKEATING, J. A., WEISS, R. A. & SATTENTAU, Q. J. (1990). Dissociation of gp120 from HIV-1 virions induced by soluble CD4. *Science* **250**, 1139–1142.

NICHOLSON, J. K. A., CROSS, G. D., CALLAWAY, C. S. & MCDOUGAL, J. S. (1986). *In vitro* infection of human monocytes with human T lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV). *Journal of Immunology* **137**, 323–329.

OU, C. Y., KWOK, S., MITCHELL, S. W., MACK, D. H., SNINSKY, J. J., KREBS, J. W., FEORINO, P., WARFIELD, D. & SCHOCHETMAN, G. (1988). DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. *Science* **239**, 295–297.

PATTERSON, S. & KNIGHT, S. (1987). Susceptibility of human peripheral blood dendritic cells to infection by human immunodeficiency virus. *Journal of General Virology* **68**, 1177–1181.

PELCHEN-MATTHEWS, A., ARMES, J. E. & MARSH, M. (1989). Internalization and recycling of CD4 transfected into HeLa and NIH3T3 cells. *EMBO Journal* **8**, 3641–3649.

PINCUS, S. H., WEHRLY, K. & CHESEBRO, B. (1991). Use of a focal infectivity assay for testing susceptibility of HIV to antiviral agents. *Bio/Techniques* **10**, 336–342.

ROBINSON, E. R., JR, MONTEFIORI, D. C. & MITCHELL, W. M. (1987). Evidence that mannosyl residues are involved in human immunodeficiency virus type (HIV-1) pathogenesis. *AIDS Research and Human Retroviruses* **3**, 265–281.

SATTENTAU, Q., DALGLEISH, A. G., WEISS, R. A. & BEVERLY, P. C. L. (1986). Epitopes of the CD4 antigen and HIV infection. *Science* **234**, 1120–1123.

SMITH, D. H., BYRN, R. A., MARSTERS, S. A., GREGORY, T., GROOPMAN, J. E. & CAPON, D. J. (1987). Blocking of HIV-1 infectivity by a soluble secreted form of the CD4 antigen. *Science* **238**, 1704–1707.

SODROSKI, J., GOH, W. C., ROSEN, C., CAMPBELL, K. & HASELTINE, W. (1986). Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. *Nature, London* **322**, 470–474.

SZOKA, F., OLSON, F., HEATH, T., VAIL, W., MAYHEW, E. & PAPAHADJOPOULOS, D. (1980). Preparation of unilamellar liposomes of intermediate size (0·1–0·2 μm) by a combination of reverse phase evaporation and extrusion through polycarbonate membranes. *Biochimica et biophysica acta* **601**, 559–571.

TATENO, M., GONZALES-SCARANO, F. & LEVY, J. A. (1989). Human immunodeficiency virus can infect CD4-negative human fibroblastoid cells. *Proceedings of the National Academy of Sciences, U.S.A.* **86**, 4287–4290.

TOYOSHIMA, K. & VOGT, P. K. (1969). Enhancement and inhibition of avian sarcoma viruses by polycations and polyanions. *Virology* **38**, 414–426.

TSCHACHLER, E., GROH, V., POPOVIC, M., MANN, D. L., KONRAD, K., SAFAI, B., ERON, L., VERONESE, F. D., WOLFF, K. & STINGL, G.

(1987). Epidermal Langerhans cells — a target for HTLV-III/LAV infection. *Journal of Investigative Dermatology* **88**, 233–237.

WEBER, J., CLAPHAM, P., MCKEATING, J., STRATTON, M., ROBEY, E. & WEISS, R. (1989). Infection of brain cells by diverse human immunodeficiency virus isolates: role of CD4 as receptor. *Journal of General Virology* **70**, 2653–2660.

WEISS, R. A., CLAPHAM, P. R., MCCLURE, M. & MARSH, M. (1989). The CD4 receptor for the AIDS virus. *Biochemical Society Transactions* **17**, 644–647.

ZACK, J. A., ARRIGO, S. J., WEITSMAN, S. R., GO, A. S., HAISLIP, A. & CHEN, I. S. Y. (1990). HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* **61**, 213–222.

ZAIA, J. A., ROSSI, J. J., MURAKAWA, G. J., SPALLONE, P. A., STEPHENS, D. A., KAPLAN, B. E., ERITJA, R., WALLACE, R. B. & CANTIN, E. M. (1988). Inhibition of human immunodeficiency virus by using an oligonucleoside methylphosphonate targeted to the tat-3 gene. *Journal of Virology* **62**, 3914–3917.

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