Synchronized Infection of Cell Cultures by Magnetically Controlled Virus

Hillel Haim,1,2* Israel Steiner,2 and Amos Panet1
Department of Virology,1 and Laboratory of Neurovirology, Department of Neurology,2 Hadassah Medical School, The Hebrew University, Jerusalem, Israel

Received 7 June 2004/Accepted 27 August 2004

To override the diffusion-limited adsorption step of viral infection, we magnetically synchronized cell attachment. Human immunodeficiency virus type 1-based lentivirus preparations were rendered magnetically reactive by association with magnetite nanoparticles, 50 nm in diameter. Application of a magnetic field resulted in immediate redistribution of the viral inoculum to the cell-associated state and completion of the productive adsorption process within 1 min. Independent of adsorption time, viral concentration, and diffusion rate, infection subsequently progressed by the receptor-mediated entry mechanism. Synchronization of this rate-limiting step of infection may now be applied to analyze isolated events in the viral replication sequence.

The rate of retroviral infection of cell cultures is primarily limited by diffusion-dependent cell association. Conforming to the diffusive properties of colloidal matter, viral particles slowly progress through the suspending solution (23). The initial cell approach is thus rendered a continuous process determined by the incubation time, mean distance from the cells, and diffusion rate (2, 3, 7). Inefficiency of the subsequent attachment step to the cell surface constitutes an additional rate-limiting factor to the progression of retroviral infection (9, 28). The resulting asynchronous nature of this initial stage in infected cell cultures hinders the analysis of both extra- and intracellular postadsorption viral events.

To overcome such limitations, several approaches aimed at increasing particle transfer to the cell surface have been utilized, including centrifugation (20) and the flow of virus through porous membranes embedded with cells (7). Such methods, while increasing the rate of cell approach to some extent, do not synchronize the adsorption step and require significant manipulation of the sample.

Magnetically reactive carriers are being increasingly harnessed for biomedical purposes. Applications include cell tracking and separation (12, 16), DNA extraction (8), viral concentration (14), and drug targeting (17). This concept has recently been utilized to augment the transfer of expression vectors for gene therapy purposes (22). Here we report the use of magnetite nanoparticles (MNP) in order to synchronize the adsorption of a viral preparation to the target cell culture.

Preparation and infection by magnetically controlled lentivirus. Primary rat aortic endothelial cells (RAECs) were isolated as previously described (19) and cultured in 12-well plates in Dulbecco’s modified Eagle’s medium supplemented with 2 mM l-glutamine, 100 U of penicillin G per ml, 0.1 mg of streptomycin per ml, and 10% fetal calf serum.

Human immunodeficiency virus type 1 (HIV-1)-based lentiviruses were generated by transient transfection of 293T cells (10). The packaging system consisted of the pCMVΔR8.91 packaging construct (29), an envelope glycoprotein construct (as noted in the text) and a pH-1-based transfer vector expressing the nuclear localized β-galactosidase or luciferase gene (18, 29). Infectious unit (IU) content of β-galactosidase-expressing lentiviral preparations was determined on RAECs in the presence of 8 µg of Polybrene per ml.

Lentiviral stocks were rendered magnetically reactive by association with anionically charged MNPs (TransMAG-PD; Chemicell, Berlin, Germany). These single-domain MNPs, 50 nm in diameter (about half the size of the lentiviral particle), are composed of an iron oxide core coated by a starch polymer modified with phosphate groups. Complexes between virus and MNP, both possessing a net negative surface charge, are formed by colloidal clustering, facilitated by positively charged ions in the solution (21, 27).

Concentrated viral stocks (24), suspended in phosphate-buffered saline (PBS) (pH 7.4), were preincubated for 2 min at room temperature with the MNP formulation (133 µg/ml in PBS) and immediately added to the cells. A magnetic field was applied by positioning a permanent Nd-Fe-B magnet (Magma Magnets, Kibbutz Gesher, Israel) under the culture plate, producing a magnetic flux density of approximately 1 T. After incubation at 37°C, the magnet was removed, and the cells were washed three times with culture medium and assayed for the HIV-1 p24 antigen as a quantitative viral marker (5) or cultured further for gene transfer assays. It should be noted that the ionic composition of both preincubation solution (PBS) and culture medium was of significant importance for virus-MNP complex formation and integrity. The level of calcium in culture medium particularly affected the stability of virus-MNP complexes, with optimal results attained at 1.5 to 2 mM CaCl2.

Synchronized adsorption of magnetically controlled virus. Magnetic field reactivity of lentiviruses pseudotyped with the vesicular stomatitis virus G protein (lenti-VSVG [6]) was assessed by measuring viral adsorption to RAEC cultures. These primary isolated cells were selected, since they may be cultured as a monolayer for several days postconfluence (19). As illus-
more than 80% of the virus preincubated with MNPs was rapidly transferred to the cell-associated state by application of a magnetic field. No significant change in viral distribution occurred during this brief adsorption period for both virus alone and virus preincubated with MNPs when no magnetic field was applied.

Deposition of the virus-MNP complexes was directly visualized by confocal laser-scanning microscopy. For this purpose, lentivirus was pseudotyped with the amphotropic murine leukemia virus 4070A envelope protein fused with the enhanced green fluorescent protein (eGFP) (25). We observed that, subsequent to the washing step of magnetically adsorbed semiconfluent RAEC cultures, viral deposition was limited to the surfaces of the cells, with no adherence to the culture dish detected.

Infectivity of magnetically adsorbed virus. The 70-fold magnetically induced increase in lenti-VSVG adsorption (Fig. 1A) correlated well with the increase in infection, as measured by both the level of transgene product (114-fold increase in luciferase activity [Fig. 1B]) and number of infection events (92-fold increase in β-galactosidase-positive cells [Fig. 1C]). We further characterized the progression of productive adsorption to the cell culture by examining the effect of incubation time on lenti-VSVG infection. As expected, the number of productive adsorption events by virus alone slowly increased, reaching maximal levels after 20 h (Fig. 2A). This increase corresponded to the decrease in residual infectivity left in the medium (Fig. 2B). In contrast, maximal productive adsorption by magnetically controlled virus was reached after 1 min of incubation. Moreover, the infectivity of magnetically adsorbed preparations was increased approximately 2.5-fold relative to the maximal values observed for virus alone (Fig. 1B, 1C, and 2A). Therefore, the plateau in infection by virus alone does not represent the complete redistribution of the inoculum to the cell-associated state but reflects the reduced infectivity of the residual virus in the medium, in accord with the previously reported half-life of 10.4 h for the VSVG-pseudotyped lentivirus (13). This was further confirmed by p24 antigen measurements, which indicated that more than 60% of the initially added virus still remained in the medium after the 20-h incubation period (data not shown). Thus, by reducing the adsorption period to 1 min, limitations imposed by particle diffusion on both cell attachment and stock infectivity were surmounted.

In the absence of a magnetic field, the infectivity of the MNP-complexed virus was significantly lower than the infectivity of virus alone (Fig. 2A). Similarly, by prolonging the preincubation period of virus with MNPs (prior to cell exposure), a steady reduction in stock infectivity was observed, as measured by both the number of infection events (β-galactosidase-positive cells) and level of transgene product (luciferase...
activity). Magnetically controlled infection of RAEC cultures with the luciferase-expressing lenti-VSVG virus preincubated with MNPs for 5, 8, and 20 min revealed 30, 44, and 57% decreases in luciferase activity levels relative to values obtained for the standard 2-min preincubation period, respectively. Prolonging the virus-MNP incubation period prior to cell attachment is therefore accompanied by a slow inactivation process, most likely by continued clustering, producing biologically inactive aggregates.

The adsorption of diffusion-controlled virus by cells is dependent upon the distance of the virus from the cell surface (2). Hence, for short incubation periods, the volume of the suspending medium affects infectivity linearly (3, 7). Indeed, as shown in Fig. 3, infection by both virus alone and virus preincubated with MNPs in the absence of a magnetic field was dependent on viral concentration. In contrast, magnetically controlled virus does not conform to Brownian diffusion and therefore demonstrated complete concentration independence.

In this report, we describe a method to override the constraints imposed by diffusion-dependent cell association on viral infection. By magnetically controlling the adsorption step, cell attachment is rendered independent of both adsorption time (Fig. 2) and mean distance from the cell monolayer (viral concentration [Fig. 3]). Furthermore, we found that adsorption is unaffected by particle diffusion rate, as determined by ambient temperature (2). While diffusion-controlled adsorption of both virus alone and MNP-complexed virus to cell monolayers was less efficient at 4 °C than at 23°C (reduced by 68 and 47%, respectively), magnetically controlled inocula were adsorbed equally at both temperatures (data not shown).

Viral host range for infection was unaltered by magnetically mediated adsorption. The β-galactosidase-expressing lentivirus was pseudotyped with the ecotropic Moloney murine leukemia virus (eco-MuLV) envelope glycoprotein, and infectivity was assessed on cell lines derived of mouse and human origin (15, 26). We found that magnetically controlled adsorption of NIH 3T3 murine cells with the eco-MuLV pseudotype significantly augmented infection; titers of viral preparations containing 1 × 10^5 IU/ml (determined by the standard serial dilution assay [24-h adsorption period]) were increased to 3.4 × 10^5 IU/ml in magnetically adsorbed cultures. However, no infection of the human-derived HeLa or 293T lines was observed in either diffusion or magnetically adsorbed cultures (titer of <10^1 IU/ml). We therefore conclude that cell entry by magnetically controlled virus is gained through the receptor-dependent mechanism.

While all experiments described in this work were performed on March 26, 2021 by guest
formed at low multiplicities of infection (MOIs of <0.01), it could be argued that due to complex formation with MNP s, viruses enter cells in clusters, potentially resulting in multiple provirus copies per cell. To test this possibility, we infected cell cultures with increasing titers (MOI of 0.01 to 4) of the eGFP-expressing lentiviral vector and examined the mean fluorescence intensity of infected cells by fluorescence-activated cell sorting analysis (11). Results indicated that, for each level of infection (percentage of eGFP-positive cells), the mean fluorescence of the eGFP-positive cell population was similar for magnetically and diffusion-controlled infection (data not shown). Therefore, a similar viral copy number infects cells adsorbed by both methods.

In conclusion, by magnetically controlling the rate-limiting adsorption step, we abolish the dependence on diffusion for viral infection. This method should prove advantageous for those applications requiring synchronous infection of a cell culture to accurately determine the kinetics of both extra- and intracellular single steps in the retroviral replication cycle.

We are grateful to Alexander Honigman and David Sanders for critical reading of the manuscript and thank Yair Ziv and Einat Tavor for technical assistance.

This work was supported by the European Commission program no. 5, Quality of Life and Management of Living Resources, and by a grant from the Philip Morris External Research Program.

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