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Efficient Gene Transduction to Cultured Hepatocytes by HIV-1 Derived Lentiviral Vector

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HEPATOCYTE transplantation can be considered a viable and effective replacement therapy for enzyme-deficient liver diseases as well as a supportive therapy for many forms of liver failure.[1] However, genetic modifications of hepatocytes in an ex vivo manner may further advance the field of hepatocyte transplantation. A proof-of-concept study by Grossman et al[2] demonstrated the clinical feasibility of transplanting ex vivo modified hepatocytes to cure inherited liver disease. Moreover, the allogeneic grafts expressed "protective genes" that could lead to immune tolerance or specific unresponsiveness in the host.

One factor critical for the success of ex vivo gene therapy is the development of a method that allows for the long-term, therapeutic expression of the transgene of interest. Among several currently available viral vector systems for use in gene transfer, retroviral vectors based on murine Moloney leukemia virus (MoLV) have been used considerably due to their ability to integrate into the host genome. This would potentially allow for the long-term transgene expression following retroviral integration.[3] However, one major drawback of MoLV is its inability to efficiently transduce genes into quiescent cells, such as primary hepatocytes in culture, due to its need for nuclear membrane dissolution.[4] Unlike MoLV, recently developed lentiviral vectors (LV) derived from HIV-1[5] have an ability to infect and transduce dividing as well as nondividing cells because of their active transport of preintegration complex through the nuclear pore.[3] We have previously reported that HIV-1 derived LV-mediated gene transduction in the liver in vivo was substantially higher than MoLV vectors.[6] In the present study, we examined the efficiency of LV-mediated transduction in cultured primary mouse hepatocytes.

MATERIALS AND METHODS

Mouse hepatocytes were isolated from C57Bl/6 mice (female, 10 to 12 weeks old) by a two-step collagenase perfusion method,[1] and subsequently purified by three rounds of low-speed centrifugation. Isolated hepatocytes were plated onto Primaria 6-well dishes (Becton Dickinson, NJ) using Williams' Medium E, which included 10% fetal bovine serum. Six hours later, the culture media was replaced with serum-free media containing a defined hormone cocktail mixture (hepatocyte growth factor [HGF] was added in some experiments). A VSV-G pseudotyped-second generation LV containing a nuclear localized lacZ cDNA driven by the murine phosphoglycerate kinase (PGK) promoter was produced and concentrated as described previously.[6,7] Twenty-four or 48-hours after plating, viral infection was performed for a period of 24 hours in the presence of polybrene (8 μg/mL) at different multiplicity of infections (MOIs). Cells were then incubated with fresh medium for 24 hours and fixed on day 3 or 4, followed by overnight staining for the presence of β-galactosidase. Positively stained nuclei were counted, and the level of transduction was expressed as a percentage of the positive X-gal stained nuclei versus the total number of hepatocytes counted. Values are shown as means $\pm$ standard deviation from three different wells in each group.

RESULTS

A dose-dependent increase in LV-mediated transduction into primary mouse hepatocytes was observed as shown in Fig 1 (viral infection was performed 24 hours after plating and cells were stained on day 3). At the highest dose of LV (MOI = 30), 61 $\pm$ 6.5% of the primary hepatocytes were transduced.

Previous studies showed that hepatocytes, which were stimulated to enter the cell cycle prior to LV administration, could significantly enhance LV transduction compared to quiescent livers in vivo.[6,7] For this reason, we investigated whether cell cycle activation of cultured hepatocytes would affect the LV-mediated ex vivo transduction efficiency. To accomplish this goal, hepatocyte growth factor (HGF), which is one of the most potent mitogens known to stimulate hepatocyte proliferation, was added to the culture media (20 ng/mL) for 42 hours prior to and for 24 hours during the addition of the LV. The presence of HGF was maintained throughout the period of vector infection (24 hours). Hepatocellular DNA replication was induced by the addition of HGF as determined by BrdU incorporation study (Fig 2B). Interestingly, the transduction efficiency was not affected by the presence of HGF at a MOI of 3 for the LV (Fig 2A).

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Supported by grant AI 41320 (MAK).

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0041-1345/02/$–see front matter
PII S0041-1345(02)02916-0

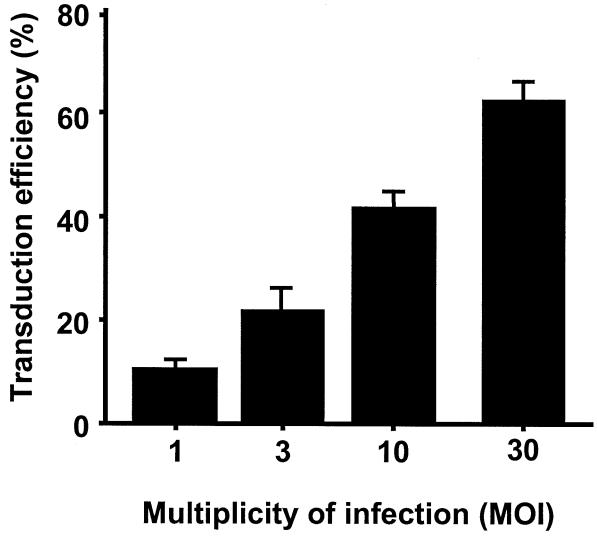

Fig 1. Lentiviral vector transduction efficiency into primary mouse hepatocytes at different multiplicity of infections. Cells were plated 24 hours before viral infection was performed for another 24 hours. The transduction efficiencies were determined on day 3.

DISCUSSION

Ex vivo gene transduction to hepatocytes prior to their implantation into a host site has been one of paradigms in hepatocyte transplantation.[1,2] Clinical trials based on the transplantation of genetically modified autologous hepatocytes have been performed in patients with familial hypercholesterolemia where MoLV vectors were used as a vehicle for the ex vivo gene transfer.[2] Although clinical safety and feasibility has been documented, this trial failed to provide convincing therapeutic effects. One plausible explanation is due to the low-level ex vivo transduction into hepatocytes achieved by MoLV.

To circumvent the problems of inefficient transduction into hepatocytes, the present study shows that HIV-1-derived LVs can efficiently transduce primary hepatocytes ex vivo. Supplemental doses of HGF in the culture medium did not affect the transduction efficiency suggesting that ex vivo gene transfer into hepatocytes using LVs may not require the use of additional proliferative agents. This finding was consistent with the report by Zahler et al,[8] where cellular proliferation was attenuated by γ-irradiation, but they showed gene transduction was unaffected using human fetal hepatocytes. Previous studies showed that in vivo LV-mediated transduction into mouse hepatocytes was significantly enhanced in the presence of hepatocellular proliferation.[6,7] The reason for the discrepancy between the ex vivo and in vivo results are not known, and further studies are needed to elucidate this difference.

Although the present study did not address the persistent level of transgene expression in the hepatocytes, we and others have shown long-term transgene expression with therapeutic efficacy in vivo.[9,10] Thus, it is reasonable to speculate that long-term transgene expression can be achieved from genetically modified hepatocytes after their transplantation into the host site as long as these cells remain viable. In all, the high level of ex vivo transduction by the hepatocytes and its potential ability for long-term transgene expression makes this vector system attractive for ex vivo gene therapy using hepatocyte transplantation.

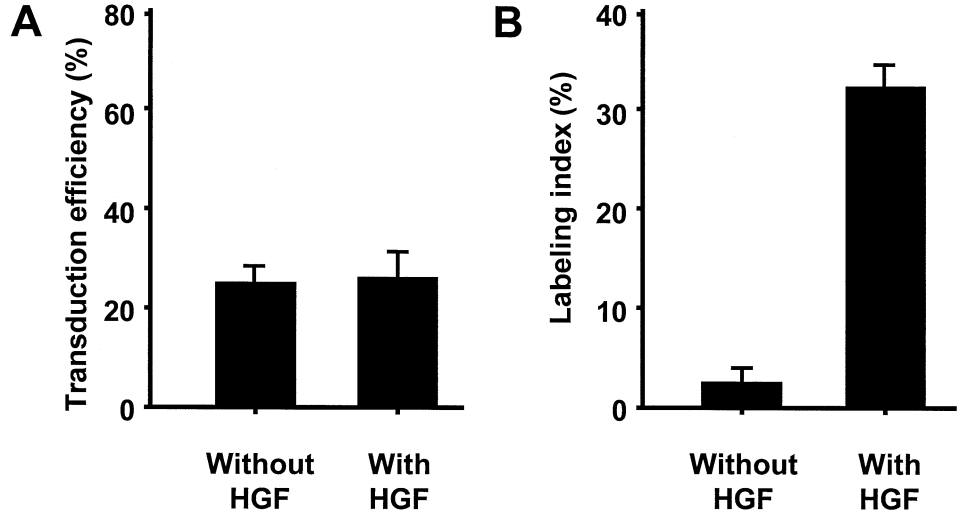

Fig 2. Effect of hepatocyte growth factor (HGF) on LV transduction efficiency **(A)** and hepatocyte DNA replication **(B)**. HGF was added at a concentration of 20 ng/mL to the culture medium 42 hours prior (starting 6 hours after plating) to the addition of the LV or BrdU and maintained following the addition of LV **(A)** or BrdU **(B)** for the 24-hour period. **A**; The hepatocytes were exposed to LV at an MOI of 3. The transduction efficiencies were determined on day 4. **B**; After the 66-hour HGF incubation period, cells were stained for BrdU by immunohistochemical method. Labeling index was determined by scoring positively stained cells.

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