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High-Efficiency Gene Transfer into CD34$^+$ Cells with a Human Immunodeficiency Virus Type 1-Based Retroviral Vector Pseudotyped with Vesicular Stomatitis Virus Envelope Glycoprotein G

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Currently, amphotropic retroviral vectors are widely used for gene transfer into CD34$^+$ hematopoietic progenitor cells. The relatively low level of transduction efficiency associated with these vectors in human cells is due to low viral titers and limitations in concentrating the virus because of the inherent fragility of retroviral envelopes. Here we show that a human immunodeficiency virus type 1 (HIV-1)-based retroviral vector containing the firefly luciferase reporter gene can be pseudotyped with a broad-host-range vesicular stomatitis virus envelope glycoprotein G (VSV-G). Higher-efficiency gene transfer into CD34$^+$ cells was achieved with a VSV-G-pseudotyped HIV-1 vector than with a vector packaged in an amphotropic envelope. Concentration of virus without loss of viral infectivity permitted a higher multiplicity of infection, with a consequent higher efficiency of gene transfer, reaching 2.8 copies per cell. These vectors also showed remarkable stability during storage at 4°C for a week. In addition, there was no significant loss of titer after freezing and thawing of the stock virus. The ability of VSV-G-pseudotyped retroviral vectors to achieve a severalfold increase in levels of transduction into CD34$^+$ cells will allow high-efficiency gene transfer into hematopoietic progenitor cells for gene therapy purposes. Furthermore, since it has now become possible to infect CD34$^+$ cells with pseudotyped HIV-1 with a high level of efficiency in vitro, many important questions regarding the effect of HIV-1 on lineage-specific differentiation of hematopoietic progenitors can now be addressed.

Gene therapy strategies to correct hematopoietic disorders in a sustained manner require transduction of therapeutic genes into stem cells (18, 21, 22). CD34$^+$ hematopoietic progenitor cells which have the capacity for pluripotential differentiation are currently being investigated as appropriate targets for gene transfer (5, 6, 23). Retroviral vectors constitute ideal vehicles for gene transfer because of their capacity to integrate into target cells and remain in the host cell chromosome to be passed on to progeny cells (18, 19). For stem cell gene therapy approaches to work effectively, it is highly desirable to transduce genes into cells with a high level of efficiency. Only moderate transduction rates, ranging from 10 to 40%, are currently possible with the present generation of murine retroviral vectors produced from packaging cell lines (5, 11–14, 20, 23). If retroviral vectors can be packaged into heterologous viral envelopes with broadened host range, higher transduction levels can be achieved as a consequence of permitting viral entry via a wider spectrum of cell surface receptors. Alternatively, increased transduction efficiency could be achieved if retroviruses could be concentrated to high titer without loss of infectivity.

A recently developed system which involves packaging of retroviral vectors into envelopes containing vesicular stomatitis virus envelope glycoprotein G (VSV-G) shows great promise (3, 31). With this system, the host range of retroviral vectors was expanded considerably to include, for example, cells of lower vertebrates such as zebra fish (16). Also, in certain human cell types like hepatocytes, higher transduction levels were achieved with the same retroviral vector backbone when pseudotyped with VSV-G compared with that packaged in an amphotropic envelope (31). Since VSV-G-containing envelopes (VSV-G envelopes) are less fragile than retroviral envelopes, pseudotyped retroviral particles can be concentrated to high titer without loss of infectivity (3). Although VSV-G-pseudotyped retroviral vectors were shown to infect cells of several species, it is not known if CD34$^+$ cells can be infected with a high level of efficiency. For stem cell gene therapy of AIDS, it is important that transduced vectors are able to work effectively in mature CD4$^+$ cells which are the main targets of human immunodeficiency virus type 1 (HIV-1). The current generation of murine retroviral vectors is expressed poorly in human lymphoid cells (18). Therefore, we considered retroviral vectors based on HIV-1 long terminal repeats for optimal expression of therapeutic genes in these cells. With these criteria in mind, we investigated three questions. First, is a retroviral vector pseudotyped with VSV-G envelope more efficient in CD34$^+$ cell gene transfer than that packaged in amphotropic envelope? Second, can transduction efficiency be increased by virus concentration? Third, can an HIV-1-based retroviral vector be packaged into VSV-G envelopes and used to infect CD34$^+$ cells? In this paper, we present data estab-

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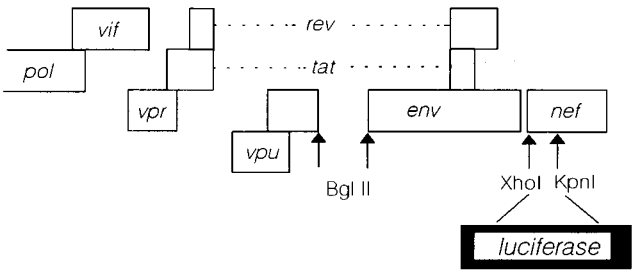

FIG. 1. Schematic representation of the 3′ portion of the envelope-defective HIV-1_{NL4-3}-based retroviral vector containing a luciferase expression marker (HIV-NL4-3 Luc). This HIV-1-based vector was generated by substituting *nef* gene sequences of the HIV-1_{NL4-3} genome with the firefly luciferase gene and deleting the envelope gene sequences located between two *Bgl*II restriction endonuclease sites as previously described (4, 25).

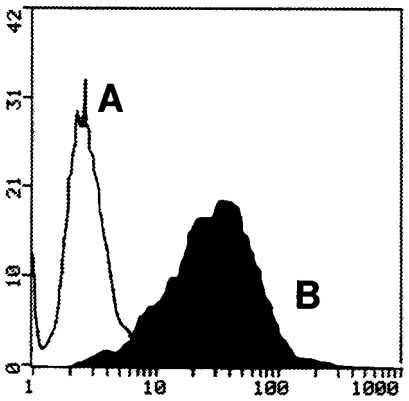

FIG. 2. Flow cytometric analysis of Cos cells expressing VSV-G. Subconfluent Cos cells were electroporated with a plasmid, pHCMV-G (3). At 24 h posttransfection, cells were incubated with a VSV-G monoclonal antibody and subsequently reacted with fluorescein isothiocyanate-labeled goat anti-mouse immunoglobulin G. The antibody-labeled cells were analyzed by flow cytometry. Peak A, negative control cells. Peak B, cells transfected with VSV-G expression plasmid.

lishing that HIV-1 genomes can be pseudotyped with VSV-G envelopes and used for high-efficiency transduction into $CD34^+$ cells.

HIV-1-based retroviral vectors can be pseudotyped with VSV-G envelope. Previous studies demonstrated that HIV-1 genomes can be packaged into heterologous retroviral envelopes, resulting in a broadened host range (28). This permitted introduction of the HIV-1 genome, albeit with moderate efficiency, into several cell types which lack the CD4 receptor. In addition, phenotypic mixing of viral envelopes is reported to occur between HIV-1 and herpesvirus, as well as VSV, in cells coinfected with these viruses (33). However, it was not clear whether the HIV-1 genome could be packaged into either herpesvirus or VSV-G envelope if these envelopes were supplied in *trans* in the absence of other herpesvirus or VSV viral proteins present during natural infection. Since pseudotyped viruses produced by this method would contain both viral populations, it would be difficult to purify them from each other. An obvious advantage in producing a pseudotyped virus which contains a single known heterologous envelope is that it would be possible to employ a single virus population rather than a heterologous mixture. To investigate the possibility of pseudotyping the HIV-1 genome with VSV-G envelope in the absence of other VSV proteins, Cos cells were cotransfected with plasmids expressing VSV-G and an envelope-defective HIV-1 genome expressing the luciferase gene (Fig. 1). Expression of VSV-G from the plasmid pHCMV-G in Cos cells was assayed at 24 h posttransfection by staining with an anti-VSV-G monoclonal antibody and by flow cytometry (3). Eighty-nine percent of the transfected cells were positive for VSV-G expression (Fig. 2). Cell culture supernatants containing the putative VSV-G-pseudotyped HIV-1 were collected at 72 h posttransfection and used to infect Cos cells. To determine evidence of HIV-1 infection, cell lysates were assayed for luciferase activity 3 days postinfection. Our results (Table 1) showed luciferase activity, indicating that the HIV-1 genome was indeed packaged into the VSV-G envelope, and the resultant pseudotyped virus was able to infect Cos cells, which are not normally susceptible to HIV-1 infection. These data established that HIV-1 can be encapsidated by VSV-G envelope when supplied in *trans* in the absence of other VSV proteins and that the resultant virus can infect cells which lack the CD4 receptor.

$CD34^+$ cells can be more efficiently transduced with a VSV-G-pseudotyped vector. To investigate whether a VSV-G-pseudotyped retroviral vector is more efficient than an amphotropic enveloped vector for $CD34^+$ cell transduction, we compared the infection efficiencies of the same HIV-1 vector backbone packaged in three distinct envelopes. The viruses contained either HIV-1 LAI envelope, amphotropic murine leukemia virus envelope, or VSV-G envelope. In a parallel series of infections, two different cell types, MT-2 and $CD34^+$ cells, were used for infection (Table 1). The multiplicity of infection was kept the same by using the same batch of viral stocks to infect both types of cells. Our results showed that HIV-1 packaged in homologous envelope LAI was able to infect MT-2 (a $CD4^+$ cell line) but not $CD34^+$ cells, whereas the same virus packaged with either amphotropic murine leukemia virus or VSV-G envelope infected both MT-2 and $CD34^+$ cells. Furthermore, the VSV-G-pseudotyped HIV-1 vector showed a 10-fold increase in $CD34^+$ cell infection compared with that for the amphotropic enveloped HIV-1 vector. This pattern of infection efficiency was consistently seen in three independent experiments (data not shown). The results presented above show that HIV-1 pseudotyped with VSV-G envelope can infect $CD34^+$ cells and does so more efficiently than pseudotyping with amphotropic envelope.

TABLE 1. Comparison of luciferase activity following infection with various pseudotyped HIV-1 vectors

Host cell and vectors	Luciferase activity (light units/ 10^5 cells)
Cos cells	
None (control uninfected cells)	108
HIV-NL4-3 Luc + pHCMV-G	64,341
MT-2 cells	
None (control uninfected cells)	107
HIV-NL4-3 Luc + pLET (HIV LAI env)[a]	4,833
HIV-NL4-3 Luc + pJD1 (amphotropic env)[a]	175,647
HIV-NL4-3 Luc + pHCMV-G (VSV-G env)[a]	180,495
$CD34^+$ cells	
None (control uninfected cells)	113
HIV-NL4-3 Luc + pLET (HIV LAI env)	191
HIV-NL4-3 Luc + pJD1 (amphotropic env)	5,204
HIV-NL4-3 Luc + pHCMV-G (VSV-G env)	54,362

[a] Construction and characterization of these envelope expression plasmids have been described previously (4, 9, 31).

TABLE 2. Luciferase activity following unconcentrated and 10×-concentrated VSV-G-pseudotyped vector infections

Host cell and virus concn	Luciferase activity (light units/10^5 cells)
MT-2 cells	
None (control uninfected cells)	131
Unconcentrated virus	742,698
10×-concentrated virus	18,290,756
$CD34^+$ cells	
None (control uninfected cells)	138
Unconcentrated virus	487,350
10×-concentrated virus	4,378,470

Transduction efficiency can be increased by concentrated virus. Virus concentration methods have been only marginally effective in increasing conventional retroviral titers, mainly because of their labile envelopes (18). In contrast, viral vectors pseudotyped with VSV-G envelope are more resistant to concentration because of the inherent stability of the viral envelope (3). To determine if the VSV-G-pseudotyped HIV-1 vector can be concentrated without loss of infectivity, ultracentrifugation was used to concentrate virus. MT-2 and $CD34^+$ cells were infected with unconcentrated and concentrated virus and assayed for luciferase activity. The viral stock used in these experiments was derived from a different transfection batch than that used for the previous experiments and showed a higher initial titer. This is reflected by higher luciferase activity of unconcentrated virus (1× virus) in Table 1 than that shown in Table 2. Our results showed that infection with 10×-concentrated virus leads to an approximately 10-fold or higher increase in luciferase activity, indicating that concentration steps did not adversely affect the virus titer (Table 2). Quantitative PCR analysis of $CD34^+$ cells infected with 1× virus revealed a 22% transduction level which increased 13-fold (2.8 copies per cell) when 10×-concentrated virus was used (Fig. 3). Similarly, MT-2 cells were transduced by 1× virus at a level of 33% with an eightfold increase in PCR signal (2.6 copies per cell) when 10×-concentrated virus was used. These results indicate that increased luciferase activity in cells infected with concentrated virus is a reflection of increased transduction levels. The data presented above suggest that it is possible to increase the viral titer of VSV-G-pseudotyped HIV-1-based vectors without loss of infectivity.

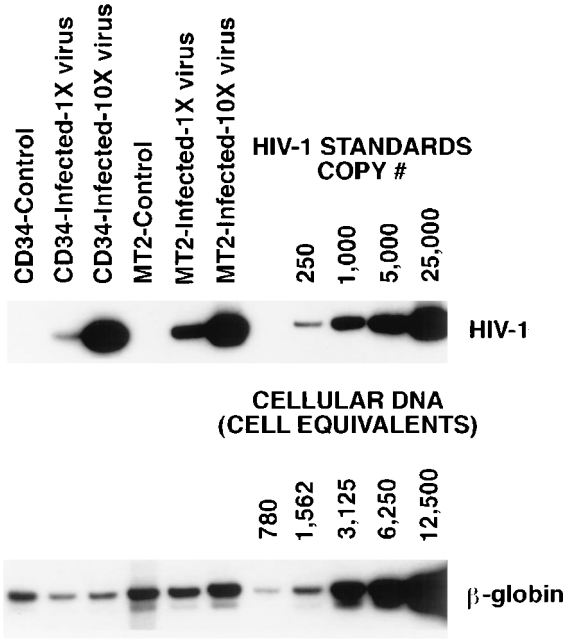

FIG. 3. Quantitative PCR analysis of vector-transduced $CD34^+$ and MT-2 cells. Cells were infected with an unconcentrated (1×) or a concentrated (10×) HIV-1-luc vector pseudotyped with VSV-G. At 3 days postinfection, DNA was extracted and analyzed by quantitative PCR for HIV-1 and human β-globin sequences as previously described (32). A set of standards run in parallel for both PCR amplifications is shown.

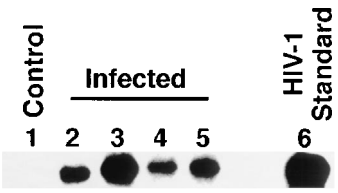

FIG. 4. PCR analysis of representative CFU-GM colonies derived from $CD34^+$ cells transduced with the HIV-1-luc vector. $CD34^+$ cells were infected with the HIV-1-luc vector as described in the text and plated in MethoCult medium (StemCell Technologies, Inc.). CFU-GM colonies were picked at day 26 postinfection. DNA was extracted and analyzed by PCR for HIV-1 sequences (32).

Progenitor cells are transduced by VSV-G-pseudotyped vectors. To determine if multipotential hematopoietic progenitor cells had been transduced by this HIV-1-luc vector and to ascertain the fate of vector sequences in lineage-committed cells, we assayed colonies of granulocytes-macrophages (CFU-GM) derived from the transduced $CD34^+$ cells for the presence of HIV-1 sequences by PCR (Fig. 4). Colonies were collected at day 26 postinfection to detect vector sequences. Our results showed the presence of the vector sequences in seven of seven CFU-GM colonies assayed, indicating that the progenitor cells were indeed transduced.

VSV-G-pseudotyped retroviral vectors are highly stable. To determine the stability of VSV-G-pseudotyped viral vectors, concentrated viral supernatants were stored under different conditions and later assayed for infectivity. Our results showed that there was no loss of infectivity during a 7-day storage either at 4°C or when frozen at −70°C and thawed (Table 3). Surprisingly, there was a slight increase in infectivity in samples stored for 7 days compared with those stored for 3 h. This phenomenon was reproducible and is possibly due to increased dissolving of the concentrated virus during storage and/or during the process of freezing and thawing.

In the present study, we showed that gene transfer into $CD34^+$ cells can be increased severalfold over that for conventional vectors by pseudotyping the retroviral vector in a VSV-G envelope. The remarkable, higher level of efficiency achieved here is likely due to the nature of the VSV-G in the envelope which seems to interact with target cells via a univer-

TABLE 3. Stability of VSV-G-pseudotyped HIV-1 vector

Storage condition (°C)	Duration	Luciferase activity (light units/10^5 cells)
4	3 h	2,310,901
−70	3 h	2,068,370
4	7 days	7,559,737
−70	7 days	4,303,680

sally present membrane component, possibly a phospholipid, in contrast to conventional, more restricted, viral ligand-cell receptor interactions (3, 17). An obvious additional advantage over standard retroviral vector systems is the ability to concentrate the vectored virus severalfold without loss of infectivity. We also showed that multiplicity of infection into $CD34^+$ cells can be increased with a corresponding increase in vector uptake and expression by the transduced cells. These two aspects of the VSV-G-pseudotyped viral vector system should facilitate high-efficiency gene transfer into $CD34^+$ cells for future gene therapy approaches. Most current protocols involve ex vivo gene transfer, in which patient cells are collected, transduced in vitro, and transferred back into the patient (18, 21, 22). Given the stability and high concentrations possible with this vector, it is conceivable that VSV-G-pseudotyped retroviral vectors may be used directly for in vivo administration without ex vivo manipulation.

Our data also established for the first time that HIV-1 genomes can be packaged into VSV-G viral envelopes via transient transfection of plasmids in the absence of coinfecting virus as seen in mixed infections. The ability to pseudotype the HIV-1 genome with VSV-G envelope, thus conferring a much broader host range, will permit many novel experiments. Many AIDS patients suffer from various hematologic cytopenias, including anemia, granulocytopenia, thrombocytopenia, and pancytopenia, suggesting that HIV-1 may infect hematopoietic progenitor cells (27). However, previous studies attempting to demonstrate that direct infection of $CD34^+$ cells by HIV-1 is responsible for these effects have been equivocal (7, 8, 10, 29). High-efficiency in vitro infection of $CD34^+$ cells with HIV-1 for hematopoietic colony assays to evaluate any deleterious effects on granulocytic or erythroid lineage-specific differentiation has not been possible previously. In the present study, we showed that HIV-1 genomes can be introduced into $CD34^+$ cells with a high level of efficiency to examine viral effects on progenitor cells.

Another potential application for this system is to determine the effect of retroviral vectors on cell lineage-specific differentiation. We have previously shown that $CD34^+$ cells can be transduced with retroviral vectors and introduced into the human thymic microenvironment in the SCID-hu mouse animal model to assess the effect of the vector sequences on $CD4^+$ and $CD8^+$ thymocyte maturation and to determine vector expression in terminally differentiated cells (1). The level of transduction efficiency, however, was low (about 10%) in these experiments. With the present VSV-G pseudotyping system, much higher transduction levels can be attained, and thus the effect and efficacy of therapeutic genes placed in $CD34^+$ cells can be assessed more quickly and with greater efficiency. One immediate application is to introduce anti-HIV-1 therapeutic genes (e.g., *trans*-dominant proteins, ribozymes, etc.) into $CD34^+$ cells (2, 15, 24, 26, 30). The transduced $CD34^+$ cells could be used to reconstitute SCID-hu mice to derive $CD4^+$ cells expressing these constructs. Subsequent challenge with virulent HIV-1 in the reconstituted SCID-hu mice will quickly determine the efficacy of anti-HIV-1 constructs in an in vivo setting.

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REFERENCES

1. **Akkina, R. K., J. D. Rosenblatt, A. G. Campbell, I. S. Y. Chen, and J. A. Zack.** 1994. Modeling human lymphoid precursor cell gene therapy in the SCID-hu mouse. Blood **84:**1393–1398.
2. **Bahner, I., C. Zhou, X.-J. Yu, Q.-L. Hao, J. C. Guatelli, and D. B. Kohn.** 1993. Comparison of *trans*-dominant inhibitory human immunodeficiency virus type 1 genes expressed by retroviral vectors in human T lymphocytes. J. Virol. **67:**3199–3207.
3. **Burns, J. C., T. Friedmann, W. Driever, M. Burrascano, and J.-K. Yee.** 1993. Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to a very high titer and efficient gene transfer into mammalian and nonmammalian cells. Proc. Natl. Acad. Sci. USA **90:**8033–8037.
4. **Camerini, D., V. Planelles, and I. S. Y. Chen.** 1994. CD26 antigen and HIV fusion? Science **264:**1160–1161.
5. **Cassel, A., M. Cottler-Fox, S. Doren, and C. E. Dunbar.** 1993. Retroviral-mediated gene transfer into CD34-enriched human peripheral blood stem cells. Exp. Hematol. **21:**585–591.
6. **Civin, C. I., and S. D. Gore.** 1993. Antigenic analysis of hematopoiesis: a review. J. Hematother. **2:**137–144.
7. **Davis, B. R., D. H. Schwartz, J. C. Marx, C. E. Johnson, J. M. Berry, J. Lyding, T. C. Merigan, and A. Zander.** 1991. Absent or rare human immunodeficiency virus infection of bone marrow stem/progenitor cells in vivo. J. Virol. **65:**1985–1990.
8. **De Luca, A., L. Teofili, A. Antinori, M. S. Iovino, P. Mencarini, E. Visconti, E. Tamburrini, G. Leone, and L. Ortona.** 1993. Haemopoietic $CD34^+$ progenitor cells are not infected by HIV-1 but show impaired clonogenesis. Br. J. Haematol. **85:**20–24.
9. **Dougherty, J. P., R. Wisniewski, S. L. Yang, B. W. Rhode, and H. W. Temin.** 1989. New retrovirus helper cells with almost no nucleotide sequence homology to retrovirus vectors. J. Virol. **63:**3209–3212.
10. **Folks, T. M.** 1991. Human immunodeficiency virus in bone marrow: still more questions than answers. Blood **77:**1625–1626.
11. **Fraser, C., C. J. Eaves, S. J. Szilvassy, and R. K. Humphries.** 1990. Expansion *in vitro* of retrovirally marked totipotent hematopoietic stem cells. Blood **76:**1071–1076.
12. **Hock, R. A., and A. D. Miller.** 1986. Retrovirus-mediated transfer and expression of drug resistance genes in human hematopoietic progenitor cells. Nature (London) **320:**275–277.
13. **Hughes, P. F. D., C. J. Eaves, S. J. Szilvassy, and R. K. Humphries.** 1989. High efficiency gene transfer to human hematopoietic cells maintained in long-term marrow culture. Blood **74:**1915–1922.
14. **Hughes, P. F. D., J. D. Thacker, D. Hogge, H. J. Sutherland, T. E. Thomas, P. M. Langdorp, C. J. Eaves, and R. K. Humphries.** 1992. Retroviral gene transfer to primitive normal and leukemic hematopoietic cells using clinically applicable procedures. J. Clin. Invest. **89:**1817–1824.
15. **Liem, D. R., A. Ramezani, X. Li, and S. Joshi.** 1993. The development of retroviral vectors expressing *trans*-dominant mutants of HIV-1 proteins to confer anti-HIV resistance. Hum. Gene Ther. **4:**625–634.
16. **Lin, S., N. Gaiano, P. Culp, J. C. Burns, T. Friedmann, J.-K. Yee, and N. Hopkins.** 1994. Integration and germ-line transmission of a pseudotyped retroviral vector in zebrafish. Science **265:**666–669.
17. **Mastromarino, P., C. Conti, P. Goldoni, B. Hauttecoeur, and N. Orsi.** 1987. Characterization of membrane components of the erythrocyte involved in vesicular stomatitis virus attachment and fusion at acidic pH. J. Gen. Virol. **68:**2359–2369.
18. **Miller, A. D.** 1992. Human gene therapy comes of age. Nature (London) **257:** 455–460.
19. **Miller, A. D., and G. J. Rosman.** 1989. Improved retroviral vectors for gene transfer and expression. BioTechniques **7:**980–990.
20. **Moore, K. A., A. B. Deisseroth, C. L. Reading, D. E. Williams, and J. W. Belmont.** 1992. Stromal support enhances cell-free retroviral vector transduction of human bone marrow long-term culture initiating cells. Blood **79:** 1393–1399.
21. **Morgan, R. A., and W. F. Anderson.** 1993. Human gene therapy. Annu. Rev. Biochem. **62:**191–217.
22. **Mulligan, R. C.** 1993. The science of gene therapy. Science **260:**926–932.
23. **Nolta, J. A., and D. B. Kohn.** 1990. Comparison of the effects of growth factors on retroviral vector-mediated gene transfer and the proliferative status of human hematopoietic progenitor cells. Hum. Gene Ther. **1:**257–268.
24. **Ojwang, J. O., A. Hampel, D. J. Looney, F. Wong-Staal, and J. Rappaport.** 1992. Inhibition of human immunodeficiency virus type 1 expression by a hairpin ribozyme. Proc. Natl. Acad. Sci. USA **89:**10802–10806.
25. **Planelles, V., F. Bachelerie, J. B. M. Jowett, A. Haislip, Y. Xie, P. Banooni, T. Masuda, and I. S. Y. Chen.** 1995. The fate of the human immunodeficiency virus type 1 provirus in infected cells: a role for *vpr*. J. Virol. **69:** 5883–5889.
26. **Sarver, N., E. M. Cantin, P. S. Chang, J. A. Zaia, P. A. Ladne, D. A. Stephens, and J. J. Rossi.** 1990. Ribozymes as potential anti-HIV therapeutic agents. Science **247:**1222–1225.
27. **Scadden, D. T., L. I. Zon, and J. E. Groupman.** 1989. Pathophysiology and management of HIV-associated hematologic disorders. Blood **74:**1455–1463.
28. **Spector, D. H., E. Wade, D. A. Wright, V. Koval, C. Clark, D. Joquish, and**

S. A. Spector. 1990. Human immunodeficiency virus pseudotypes with expanded cellular and species tropism. J. Virol. **64:**2298–2308.
29. **Stanley, S. K., S. W. Kessler, J. S. Justement, S. M. Shnittman, J. J. Greenhouse, C. C. Brown, L. Musongela, K. Musey, B. Kapita, and A. S. Fauci.** 1992. CD34+ bone marrow cells are infected with HIV in a subset of seropositive individuals. J. Immunol. **149:**689–697.
30. **Vandendriessche, T., M. K. L. Chuah, L. Chiang, H.-K. Chang, B. Ensoli, and R. A. Morgan.** 1995. Inhibition of clinical human immunodeficiency virus (HIV) type 1 isolates in primary $CD4^+$ T lymphocytes by retroviral vectors expressing anti-HIV genes. J. Virol. **69:**4045–4052.
31. **Yee, J.-K., A. Miyanohara, P. LaPorte, K. Bouic, J. C. Burns, and T. Friedmann.** 1994. A general method for the generation of high-titer, pantropic retroviral vectors: highly efficient infection of primary hepatocytes. Proc. Natl. Acad. Sci. USA **91:**9564–9568.
32. **Zack, J. A., S. J. Arrigo, S. Weitsman, A. S. Go, A. Haislip, and I. S. Y. Chen.** 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. Cell **61:**213–222.
33. **Zhu, Z., S. S. L. Chen, and A. S. Huang.** 1990. Phenotypic mixing between human immunodeficiency virus and vesicular stomatitis virus or herpes simplex virus. J. Acquired Immune Defic. Syndr. **3:**215–219.