Proc. Natl. Acad. Sci. USA
Vol. 87, pp. 4349–4353, June 1990
Medical Sciences

Expression of human α-globin and mouse/human hybrid β-globin genes in murine hemopoietic stem cells transduced by recombinant retroviruses

(retroviral vectors/gene therapy/bone marrow transplantation)

C. L. Li*, V. J. Dwarki, and I. M. Verma†

Molecular Biology and Virology Laboratory, The Salk Institute, P.O. Box 85800, San Diego, CA 92138

Communicated by Renato Dulbecco, March 12, 1990 (received for review January 16, 1990)

ABSTRACT **Murine cell lines releasing helper-free recombinant retroviruses containing human α-globin and mouse/human hybrid β-globin genes were generated. The expression of the hybrid β-globin gene but not the human α-globin gene was regulated appropriately in infected mouse erythroid leukemia (MEL) cells. Murine bone marrow cells were infected by coculture with virus-producing cells and transplanted into lethally irradiated syngeneic recipients. Greater than 90% of the spleen colonies (12–15 days), which are derived from hemopoietic multipotential stem cells, showed proviral integration. Various levels of expression of the transduced globin genes were detected in all of the provirus-positive spleen colonies. Proviral sequences and transcripts from the transduced globin genes could also be detected in a few long-term reconstituted recipients in an observation period of 10 months after transplantation.**

In the past few years retroviral vectors have increasingly been used to introduce foreign genes into cells, tissues, and whole animals. The ability to generate high-titer, helper-free recombinant retroviruses, combined with their ability to infect a large variety of cell types, has contributed to their popularity as versatile vectors for gene transfer. Many investigators have entertained the notion of using recombinant retroviruses as primary vehicles for introducing genes into cells, with a view toward somatic cell gene therapy. One of the tissues often considered for gene therapy is the bone marrow because of the presence of pluripotent stem cells capable of proliferation and differentiation during the entire life span of an individual. Work from many laboratories using recombinant retroviruses to infect pluripotent stem cells suggests that (*i*) it is possible to efficiently infect mouse bone marrow cells and (*ii*) upon reconstitution of the infected bone marrow cells, the expression of foreign genes carried by means of the retroviral vectors is generally very poor (1–11).

We have been interested in introducing human α- and β-globin genes into mouse bone marrow cells with the ultimate goal of demonstrating the feasibility of somatic gene therapy for human hemoglobinopathies. In this communication we report the construction and expression of high-titer retroviral vectors containing human α-globin and mouse/human hybrid β-globin genes in blood cells following transplantation of infected bone marrow cells in lethally irradiated mice. We show low but sustained expression (5 months) in long-term reconstitution experiments.

MATERIALS AND METHODS

Animals, Cell Culture, and Virus Infection. Adult male and female C57BL/6J mice (6–8 weeks old) were obtained from The Jackson Laboratory. All the cell lines (12–15) except the mouse erythroid leukemia (MEL) cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (vol/vol) calf serum (HyClone) in a 10% CO_2 atmosphere. MEL cells were maintained in DMEM with 15% (vol/vol) horse serum. The generation of virus-producing cell clones releasing ecotropic recombinant retroviruses and the virus titer assay were carried out essentially as described (16). Helper virus was assayed by the marker rescue method (3). The concentration of G418 (GIBCO) used to select for neomycin-resistant (Neo^R) cells in the viral assays was 400 μg/ml (active). For induction of differentiation in MEL cells, dimethyl sulfoxide (DMSO) was included in the growth medium at a final concentration of 1.8% for 4 days.

Plasmids and Vector Construction. To construct α- and β-globin recombinant viruses, various DNA fragments of the globin genes were individually inserted into the unique *Xho* I site of the N2 vector containing the bacterial neomycin phosphotransferase gene (17). The 1.5-kilobase (kb) *Pst* I human α-globin DNA fragment was derived from the plasmid $pSv_o\alpha 1p3d$; the 4.4-kb *Pst* I and the 4.1-kb *Hin*cII–*Xba* I human β-globin DNA fragments were derived from plasmids pSP65BPst(+) and pHβ8, respectively, whereas the 2.3-kb *Cla* I–*Xho* I mouse/human hybrid β-globin DNA fragment was derived from pMHβ (18). The DNA fragment contains the 5′ portion of the mouse β^{maj}-globin gene (−106 nucleotides upstream of the cap site) and the 3′ portion of the human β gene (+2200) fused at a conserved *Bam*HI site in the second exon of both genes. In addition, the 5′ untranslated region of the hybrid β-globin gene contains a 12-base-pair insert at position +28 to discriminate between accurately initiated transduced and mouse endogenous β-globin genes.

Bone Marrow Cell Infection. Bone marrow cells flushed from the femurs and tibia of adult 8- to 14-week-old C57BL/6J mice were collected and cocultivated with irradiated (20 grays) virus-producing cells plated 24 hr previously in DMEM supplemented with 20% fetal calf serum, 10% WEHI-3B conditioned medium, and Polybrene (Aldrich) at 6 μg/ml. Two days after cocultivation, G418 was added to a final concentration of 2 mg/ml (active substance), and the cultures were incubated further for 48 hr before harvest. The supernatant containing the nonadherent bone marrow cells was collected and replated to remove any adhering fibroblast cells. The bone marrow cells were injected by means of the tail vein into syngeneic mice that had received a single dose of irradiation (9.5 grays from a cobalt source). For the isolation of individual spleen colonies, 10^4–10^5 cells were injected, whereas 10^5–10^6 cells were used to generate long-term reconstituted mice. Peripheral blood (100-μl) samples

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LTR, long terminal repeat; DMSO, dimethyl sulfoxide; Neo^R, neomycin resistant; nt, nucleotide.

*Present address: The Walter and Eliza Hall Institute, Post Office, Royal Melbourne Hospital, Victoria 3050 Australia.
†To whom reprint requests should be addressed.

were collected from the tail vein at different time intervals after transplantation for DNA and RNA analysis. In some experiments, spleen fragments were collected after partial splenectomy. Eight to 10 months after transplantation, all recipient mice were sacrificed; single-cell suspensions from bone marrow, spleen, thymus, and mesenteric lymph nodes were prepared for DNA and RNA analysis by standard recombinant DNA procedures.

RESULTS

Generation of Recombinant Retroviruses. The structure of the various constructs containing human α- and β-globin genes and mouse/human hybrid β-globin genes, the titer of the recombinant retroviruses produced by each Neo^R colony, and the frequency of the intact proviruses in the Neo^R colonies are shown in Fig. 1. The following general conclusions can be made: (*i*) the percentage of Neo^R colonies generating high-titer recombinants (>10^5 virus particles per ml) is independent of the orientation of the globin genes with respect to the promoter in the viral LTR, (*ii*) the intact proviral DNA structure is maintained only when the promoter of the globin genes is inserted in an orientation opposite to that of the viral long terminal repeat (LTR) (referred to as "−" in Fig. 1), (*iii*) no recombinant retroviruses containing intact human β-globin gene could be generated (0 out of 57) as compared to the mouse β^{maj}/human β-globin chimeric gene (24 out of 26), and (*iv*) generally the N2 vector/ψ2 cell-generated retroviruses had low levels of helper virus.

Expression of Human α-Globin in Spleen Colonies. Fresh bone marrow cells were infected with high-titer human α-globin recombinant viruses and after appropriate manipulations (see *Materials and Methods*) were injected into lethally irradiated syngeneic recipients. After 15 days of reconstitution, individual spleen colonies were isolated and analyzed for the presence of a 4.7-kb diagnostic fragment of the proviral DNA. Greater than 90% of the colonies showed intact proviral DNA (data not shown). RNA was isolated from individual colonies and analyzed by RNase protection for the expression of human α-globin mRNA. As shown in Fig. 2*A*, most of the spleen colonies exhibited a protected 133-nucleotide (nt) α-globin mRNA-specific fragment. The level of expression varied between colonies and generally represented only 0.1–1% of the endogenous mouse α-globin RNA (compare with Fig. 2*B*; nearly 20 times less RNA was used in Fig. 2*B*).

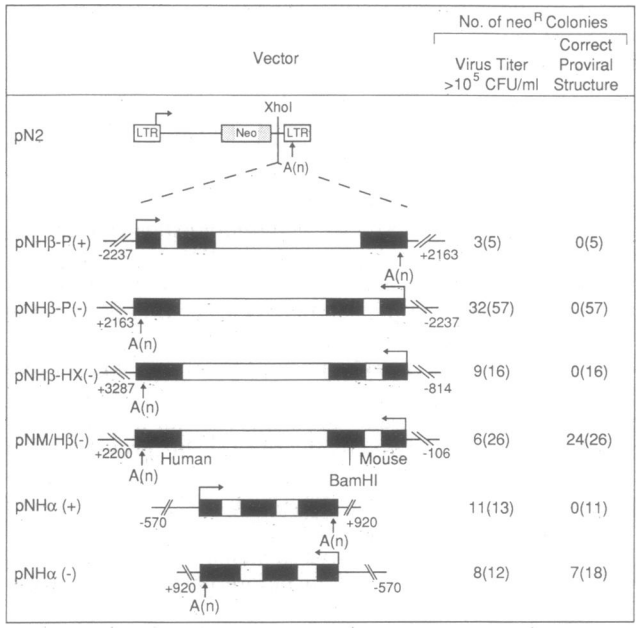

FIG. 1. Structure of the various constructs, titers of the recombinant retroviruses, and integrity of provirus DNA in various constructs. For each measurement, the number of Neo^R colonies examined is in parentheses. The presence of an intact provirus structure was based on Southern blot analysis. pN2, N2 vector; pNMβ-P, human β-globin gene *Pst* I fragment; pNMβ-HX, human β-globin *Hin*cII–*Xba* I fragment; pNM/Hβ, 5′ mouse β^{maj}/human 3′ hybrid β-globin gene; pNHα, human α-globin gene fragment; +, orientation of the promoter of globin gene in the same orientation as the mouse LTR promoter; −, opposite orientation of the globin gene promoter as compared to LTR; CFU, colony-forming unit. Filled boxes indicate the locations of exons; the bent arrows indicate the promoters; vertical arrows indicate poly(A) addition signals.

A total of 25 long-term reconstituted mice were analyzed for human α-globin mRNA transcripts in the peripheral blood at different time intervals after transplantation. Low levels of expression of human α-globin transcripts could be observed in two mice (Fig. 2*C*). In mouse no. 1 the human α-globin gene transcripts could be detected up to 5 months after transplantation (Fig. 2*C*, lanes 1, 5, and 9), and in mouse no. 2 it was detected only at the fourth month after transplantation (Fig. 2*C*, lane 6). In a parallel experiment, partial splenectomy was performed at various times after reconstitution, and Southern blot analysis of the genomic DNA from spleens of five mice showed that two mice were positive for proviral integration. In one mouse the proviral DNA was detected after 3 months, whereas in the other the proviral DNA was evident only after 6 months (data not shown). To further analyze the expression of the human α-globin gene in these two mice, various organs were collected for DNA and RNA analysis. Furthermore, spleen cells were crudely separated by panning into B cells and T cells. RNase protection analysis of the α-globin RNA transcripts showed that one mouse that had been reconstituted for 3 months expressed low levels in B cells and lymph nodes (Fig. 2*C*, lanes 14 and 15). In the second mouse, no expression was detectable. We conclude that human α-globin gene transcripts can be observed in peripheral blood after transplantation of infected bone marrow cells, but the levels are very low and are not regulated in a tissue-specific manner.

Regulated Expression of the Mouse β^{maj}/Human β-Globin Hybrid Gene. Since the expression of the human α-globin gene is not regulated, we wanted to determine if cells infected with the recombinant retroviruses containing the mouse/human hybrid β-globin gene show regulated expression. It has previously been shown that sequences in the 5′ and in the intron of the β-globin gene are required for its erythroid-specific expression (19, 20). We therefore infected MEL cells, which upon treatment with chemical inducers can undergo a coordinate series of morphological and biochemical changes mimicking normal adult erythroid cell differentiation. G418-resistant MEL cells were isolated and induced with DMSO, and total cellular RNA was isolated from induced and uninduced cells. The probes used can identify a protected 152-nt fragment diagnostic of the mouse/human hybrid gene and a 118-nt fragment corresponding to endogenous mouse β^{maj}-globin gene. Analysis of uninfected control MEL cells showed a series of fragments of ≈118 nt in length that increased at least 10-fold after induction with DMSO (Fig. 3*A*, lanes 1 and 2). In infected MEL cells an additional band of 152 nt representing the mouse/human hybrid gene was detected upon induction with DMSO (compare lanes 3 and 4 in Fig. 3*A*). In clones 27 and 31, the degree of induction of mouse/human hybrid genes appears to be comparable to that observed with the endogenous mouse β-globin gene. To determine whether transcripts from the transduced hybrid β-globin gene terminated correctly, a β-globin gene probe specific for the 3′ portion of the human β-globin gene was used. As shown in Fig. 3*B*, only in infected MEL cells, a series of 210- to 212-nt protected fragments inducible with DMSO treatment can be observed.

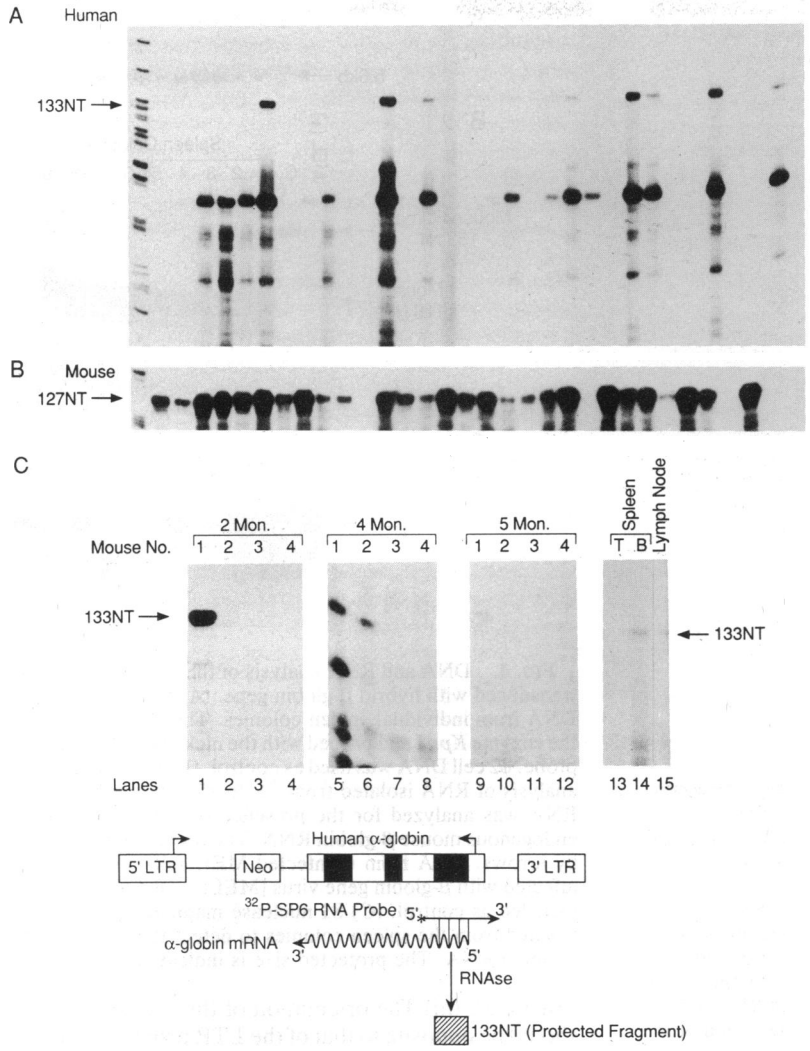

FIG. 2. Expression of human α-globin RNA. (*A* and *B*) Ribonuclease protection analysis of total RNA isolated from 12- to 15-day spleen colonies. (*A*) Five micrograms of total RNA was analyzed for the presence of human α-globin RNA. (*B*) One-fourth microgram of total RNA was used for analysis of mouse α-globin RNA. The protected size fragment is indicated by an arrow. (*C*) Ribonuclease protection of total RNA isolated from peripheral blood samples collected at different time points as indicated above the autoradiogram (lanes 1–12). Lanes 13–15 contain RNA isolated from T cells, B cells, and lymph node cells from a long-term reconstituted mouse (3 months). Total RNA was isolated from 100 μl of peripheral blood and from the tissues indicated and analyzed for human α-globin mRNA. The protected fragment is indicated by an arrow. The structure of the human α-globin RNA probe and the size of the protected fragment are indicated in the diagrammatic sketch.

Mouse/Human Hybrid β-Globin Gene Expression in Spleen Colonies. In experiments analogous to those performed with the human α-globin gene recombinant retroviruses (Fig. 2), we cocultivated fresh mouse bone marrow cells with ψ2 cells producing mouse/human β-globin retrovirus. Fig. 4*A* shows that all the spleen colonies contained 1–3 copies of unrearranged β-globin proviruses as judged by the diagnostic 5.6-kb DNA fragment. RNase protection analysis indicated that, in addition to the endogenous mouse β-globin-protected fragment (118 nt), an additional protected fragment of 152 nt, corresponding to the mouse/human hybrid β-globin gene, was present in the RNA obtained from the spleen colonies (Fig. 4*B*). The relative signal intensity of the two protected fragments (152 and 118 nt) indicated that the retrovirally transduced hybrid β-globin gene is expressed at <0.1% of the level of the mouse endogenous β^{maj}-globin gene in the spleen colonies. Furthermore, the level of expression of the transduced hybrid β-globin varied between individual spleen colonies and had no apparent correlation with the proviral copy number. Similar results were obtained when a 3′ probe specific for the human β-globin gene was used as a probe (Fig. 4*C*).

Long-Term Expression of the Mouse/Human Hybrid β-Globin Gene. Peripheral blood samples were collected at different time intervals after transplantation and analyzed for evidence of proviral integration and expression of the transduced hybrid β-globin gene. Fig. 5 shows such an analysis of seven animals 3 weeks after transplantation. Nuclease S1 protection analysis of RNA prepared from peripheral blood cells revealed that six out of seven animals showed significant though varying levels of hybrid β-globin gene expression. However, by 4 months after transplantation, only one mouse (no. 10, Fig. 5*B*) showed evidence of low-level expression of the hybrid β-globin gene. Two recipients were identified to contain cells harboring proviral sequences in their blood samples throughout the period of observation of 10 months after transplantation, indicating that the hemopoietic stem cells have been infected with the recombinant retroviruses. However, no hybrid β-globin transcripts could be detected in these mice. Ten months after transplantation, one mouse was sacrificed and DNA and cytoplasmic RNA from various hemopoietic organs were extracted for analysis. In this mouse, proviral sequences could be detected in spleen, in mesenteric lymph node cells, and to a lesser extent in the bone marrow cells and thymocytes (Fig. 5*C*), but no hybrid β-globin message could be detected.

Finally, to determine whether the "infected" bone marrow cells after the preselection phase successfully engrafted the lethally irradiated recipients, bone marrow cells from male mice were used to reconstitute female recipients in a series of experiments. By using a Y chromosome-specific probe, it was determined that all recipients (5 out of 5) receiving unselected (no G418 preselection) infected bone marrow cells contained a significant number of donor cells in their peripheral blood 6 months after transplantation. However, no or few donor cells were found in the peripheral blood in a large proportion (6 out of 10 and 5 out of 7 animals in two separate experiments) of mice receiving preselected infected bone marrow cells, presumably due to loss of progenitor cells during G418 selection.

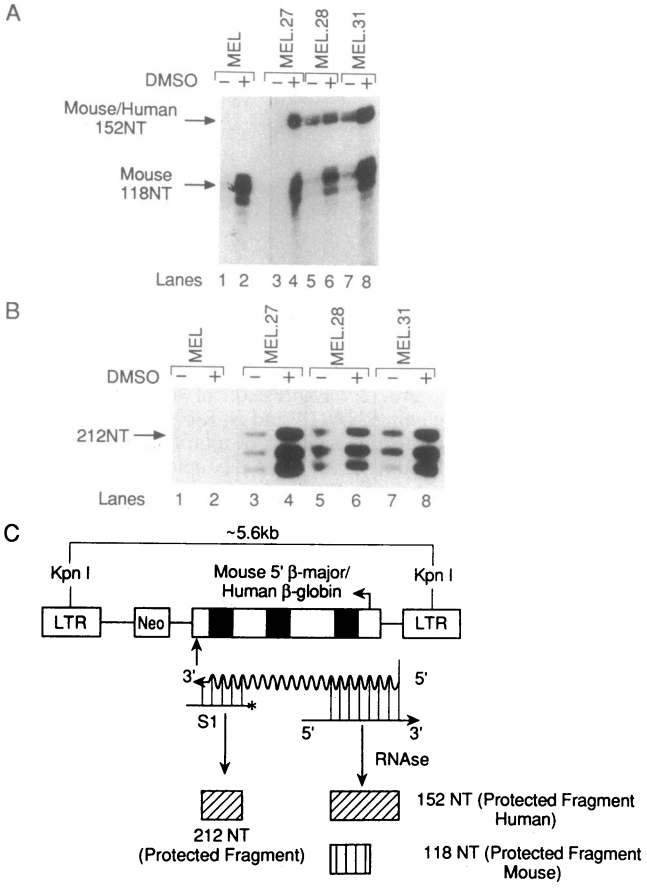

FIG. 3. Expression of hybrid β-globin RNA in MEL cells. (*A*) Hybrid β-globin RNA was measured in induced and uninduced MEL cells by RNase protection analysis. Total RNA was isolated from pools of G418-resistant cells resulting from infection with the hybrid β-globin amphotrophic virus. (*B*) The same samples of RNA used in *A* were assayed by S1 nuclease mapping to detect the 3′ end of the hybrid β-globin RNA. (*C*) The structure of the hybrid β-globin gene and the probes used in the experiment are indicated in the diagrammatic sketch.

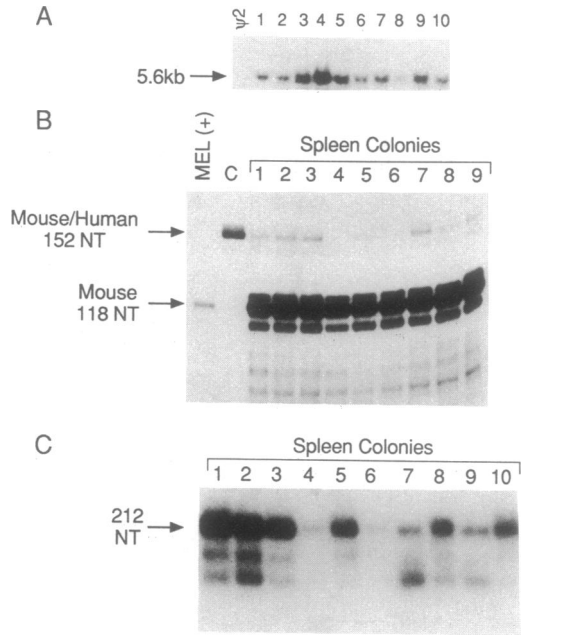

FIG. 4. DNA and RNA analysis of the individual spleen colonies transduced with hybrid β-globin gene. (*A*) Southern blot analysis of DNA from individual spleen colonies. The DNA was digested with the enzyme *Kpn* I and probed with the nick-translated neomycin gene probe. ψ2 cell DNA was used as control. (*B*) Ribonuclease protection analysis of RNA isolated from 12- to 15-day spleen colonies. Total RNA was analyzed for the presence of both hybrid β-globin and endogenous mouse β-globin RNA. The protected sizes are indicated by arrows. RNA from uninfected MEL cells and from MEL cells infected with β-globin gene virus [MEL(+) and C, respectively] was included as control. (*C*) S1 nuclease mapping of the RNA samples isolated from the spleen colonies to detect the 3′ end of the hybrid β-globin RNA. The protected size is indicated by an arrow.

DISCUSSION

Our studies show that human α-globin gene can be transduced and expressed in murine hematopoietic stem cells. We also show that retroviral constructs containing the hybrid mouse/human β-globin gene are more stable than the complete human β-globin gene. Our work also demonstrates that retrovirally transduced genes can be detected for at least 10 months after reconstitution of infected bone marrow progenitor cells.

We had three principal goals: (*i*) generation of high-titer recombinant retroviruses with unaltered genes, (*ii*) efficient introduction into progenitor cells, and (*iii*) monitoring expression in recipients after long-term reconstitution. This is particularly important when the constructs contain more than one gene. Our data utilizing a selectable (Neo^R) and a nonselectable (globin) gene support the following general observations. (*i*) The titer of the recombinant retroviruses differs in different clones. The exact reason for this heterogeneity, which can vary between 10^2 and 10^6 virus particles, remains obscure, but from practical considerations, it is important to screen individual clones for the titer of recombinant viruses. (*ii*) In constructs containing more than one gene construct, the integrity of the individual genes (i.e., correct proviral structure) should be ascertained. As shown in Fig. 1, the α- and β-globin gene proviruses undergo alterations even though high-titer Neo^R retroviruses can be generated. (*iii*) The orientation of the second gene promoter should be opposite to that of the LTR promoter. Only in those constructs where the globin gene is in the opposite orientation could correct proviral structures be identified. This observation is in agreement with those previously reported for globin and other genes (5–7, 9). (*iv*) The titers of the recombinant viruses are not influenced by the orientation of the genes in the retroviral constructs. (*v*) Some genes have sequences that interfere with the generation of intact proviruses. As shown in Fig. 1, no recombinants could be obtained with the intact human β-globin gene [pNHβ-P(−) or pNβ-HX(−)]. However, replacement of the 5′ region of the human β-globin gene with the mouse β^{maj}-globin gene allows the generation of recombinants with intact proviruses. These results are in agreement with Miller *et al.* (21) who showed that removal of sequences 5′ to intron 2 in the human β-globin gene generated recombinants with intact proviruses.

The regulation of the β-globin gene has been well documented and requires sequences in intron 2 (19, 21). We show that retroviruses containing the mouse/human hybrid β-globin gene can be appropriately regulated in MEL cells (Fig. 3 *A* and *B*). Thus it is likely that these recombinant retroviruses will maintain their erythroid expression in intact animals as has been shown by Dzierzak *et al.* (5). In contrast, the human α-globin gene is not expressed in a tissue-specific manner. Unlike β-globin retroviruses, the fibroblasts infected with α-globin retroviruses produce copious amounts of α-globin mRNA. Furthermore, in long-term reconstituted mice (Fig. 2*C*), human α-globin transcripts could be detected in B cells and lymph nodes (Fig. 2*C*). Recently, a number of investigators have identified a locus activation region (LAR) nearly 60 kb upstream of the β-globin locus that, once linked to β-globin

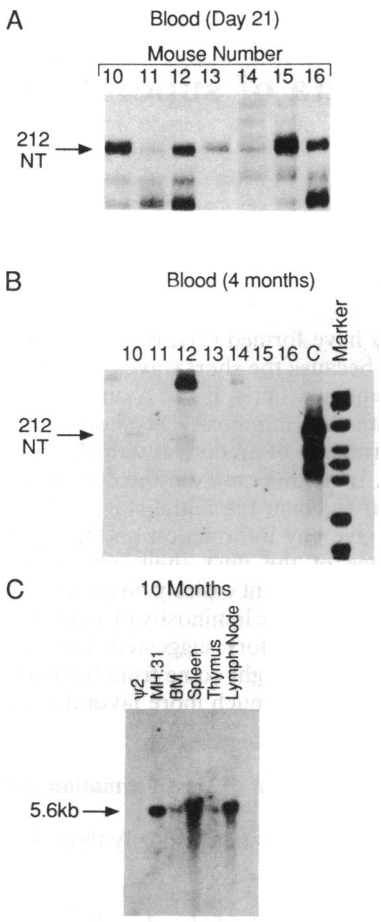

FIG. 5. Expression of hybrid β-globin RNA in long-term reconstituted mice. (*A* and *B*) S1 nuclease mapping of the RNA isolated from peripheral blood samples of the β-globin transduced mice at the indicated time points. Lane C in *B* contains an RNA sample from DMSO-induced MEL cells infected with the hybrid β-globin gene virus. (*C*) Southern blot analysis of DNA isolated from one of the long-term reconstituted mice. A 5.6-kb expected band is indicated by an arrow. The ψ2 lane contains a DNA sample isolated from ψ2 cells, and the MH.31 lane contains DNA isolated from an ecotropic hybrid β-globin virus-producing clone.

gene, enhances expression in a tissue-specific manner (22–30). Perhaps some of these sequences in retroviruses containing the α-globin gene may allow erythroid specificity.

Several investigators have shown that mouse pluripotent stem cells can be infected with recombinant retroviruses (1–10). Presently the major problems are (*i*) the requirement of high-titer recombinant retroviruses (usually greater than 10^5 virus particles per ml), (*ii*) detection of recombinant proviral DNA in the peripheral blood cells for a sustained period in only some lineages, and (*iii*) parsimonious expression of the recombinant genes. Additionally, our results presented here show that, although the inclusion of preselection with G418 following cocultivation enriched the proportion of colony-forming units–spleen harboring proviral sequences, most of these cells apparently do not have self-renewal capacity. Using the Y chromosome-specific probe to differentiate donor and recipient cells, we showed that bone marrow cells after G418 selection apparently did not engraft the lethally irradiated recipients as efficiently as the unselected cells. A similar observation has been reported in studies using W/W^v mice (31). Recently, Bodine *et al.* (32) and Correll *et al.* (33) have shown that the combination of interleukins 3 and 6 in the culture medium increases the frequency of retroviral gene transfer by increasing the number of stem cells in the cycle. Alternatively, purification of stem cells prior to infection seems to increase the population of stem cells infected as shown recently (34). Redesign of retroviral vectors for the generation of high-titer globin-containing viruses and improvement in culture conditions for high efficiency of viral infections and maintenance of the long-term repopulating capability of the infected stem cells are necessary for effective retrovirus-mediated gene transfer for gene therapy.

We are grateful to Dr. P. Mellon for help and for providing many reagents during the course of this work. We thank Dr. E. Gilboa for providing the N2 vector and Dr. R. Mulligan for the ψ2 cells. We also thank Ms. Dana Rashid for excellent technical help and Ms. Pat McClintock for typing the manuscript. C.L.L. was supported by a postdoctoral fellowship from the American Chemical Society (F#J-32-86). This work was supported by Grant R1HL37072A from the National Heart, Lung, and Blood Institute.

1. Miller, A. D., Eckner, R. J., Jolly, D. J., Friedman, T. & Verma, I. M. (1984) *Science* **225,** 630–632.
2. Williams, D. A., Lemischka, I. R., Nathans, D. G. & Mulligan, R. C. (1984) *Nature (London)* **310,** 476–480.
3. Keller, G., Paige, C., Gilboa, E. & Wagner, E. F. (1985) *Nature (London)* **318,** 149–154.
4. Dick, J. E., Magli, M. C., Huszar, D. H., Phillips, R. A. & Bernstein, A. (1985) *Cell* **42,** 71–79.
5. Dzierzak, E. A., Papayannoupoulou, T. & Mulligan, R. C. (1988) *Nature (London)* **331,** 35–41.
6. Karlsson, S., Bodine, D. M., Perry, L., Papayannoupoulou, T. & Nienhuis, A. W. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6062–6066.
7. Belmont, J. W., MacGregor, G. R., Wagner-Smith, K., Fletcher, F. A., Moore, K. A., Hawkins, D., Willalon, D., Chang, S. M.-U. & Caskey, C. T. (1988) *Mol. Cell. Biol.* **8,** 5116–5125.
8. Wong, P. M. C., Chung, S. W., Dunbar, C. E., Bodine, D. M., Ruscetti, S. & Nienhuis, A. W. (1989) *Mol. Cell. Biol.* **9,** 798–808.
9. Johnson, G. R., Gonda, T. J., Metcalf, D., Hariharan, I. & Cory, S. (1989) *EMBO J.* **8,** 441–448.
10. Eglitis, M. A., Kantoff, P., Gilboa, E. & Anderson, W. F. (1985) *Science* **230,** 1395–1398.
11. St. Louis, D. & Verma, I. M. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 3150–3154.
12. Wei, C.-M., Gibson, M., Spear, P. G. & Scolnick, E. M. (1981) *J. Virol.* **39,** 935–944.
13. Ralph, P. & Nakoinz, I. (1977) *Cancer Res.* **37,** 546–550.
14. Miller, A. D., Law, M. D. & Verma, I. M. (1984) *Mol. Cell. Biol.* **5,** 431–437.
15. Mann, R., Mulligan, R. C. & Baltimore, D. (1983) *Cell* **33,** 153–159.
16. Miller, A. D., Trauber, D. R. & Buttimore, C. (1986) *Somatic Cell. Mol. Genet.* **12,** 175–183.
17. Armentano, D., Yu, S.-F., Kantoff, P. W., von Ruden, T., Anderson, W. F. & Gilboa, E. (1987) *J. Virol.* **61,** 1647–1650.
18. Chao, M. V., Mellon, P., Charnay, P., Maniatis, T. & Axel, R. (1983) *Cell* **32,** 483–493.
19. Hamer, D. & Leder, P. (1979) *Cell* **18,** 1299–1302.
20. Rutherford, T. & Nienhuis, A. W. (1987) *Mol. Cell. Biol.* **7,** 398–402.
21. Miller, A. D., Bender, M. A., Harris, E. A. S., Kaleko, M. & Gelinas, R. E. (1989) *J. Virol.* **62,** 4337–4345.
22. Hanscombe, O., Vidal, M., Kaeda, J., Luzzatto, L., Greaves, D. R. & Grosveld, F. (1989) *Genes Dev.* **3,** 1572–1581.
23. Spandidos, D. A. & Paul, J. (1982) *EMBO J.* **1,** 15–20.
24. Wright, S., Rosenthal, A., Flavell, R. & Grosveld, F. (1984) *Cell* **38,** 265–273.
25. Constantini, F., Chada, K. & Magram, J. (1986) *Science* **233,** 1192–1194.
26. Townes, T. M., Lingrel, J. B., Chen, H. Y., Brinster, R. L. & Palmiter, R. D. (1985) *EMBO J.* **4,** 1715–1723.
27. Tuan, D., Solomon, W., Qiliang, L. S. & Irving, M. L. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 6384–6388.
28. Forrester, W. C., Takegawa, S., Papayannoupoulou, T., Stamatoyannopoulos, G. & Groudin, M. (1987) *Nucleic Acids Res.* **15,** 10159–10177.
29. Grosveld, F., Van Assendelft, G. B., Greaves, D. R. & Kollias, G. (1987) *Cell* **51,** 975–985.
30. Behringer, R. R., Hammer, R. E., Brinster, R. L., Palmiter, R. D. & Townes, T M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 7056–7060.
31. Bender, M. A., Gelinas, R. E. & Miller, A. D. (1989) *Mol. Cell. Biol.* **9,** 1426–1434.
32. Bodine, D. M., Karlsson, S. & Nienhuis, A. W. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8897–8901.
33. Correll, P. H., Fink, J. K., Brady, R. O., Perry, L. K. & Karlsson, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8912–8916.
34. Szilvassy, S. J., Fraser, C. C., Eaves, C. J., Lansdorp, P. M., Eaves, A. C. & Humphries, R. K. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 8798–8802.