Manipulation of Mouse Hematopoietic Progenitors by Specific Retroviral Infection*

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Previous studies have identified an enhancer 3' of the scl gene that can direct transgene expression to hematopoietic progenitors and stem cells. Here we use this enhancer to restrict expression of the avian leukemia virus receptor, TVA, to hematopoietic stem cells and progenitors in bone marrow and fetal liver and demonstrate that retroviral infection can be used to specifically introduce exogenous sequences. We show that a majority of CFU-S12 multipotential progenitor cells can be transduced in vitro. Uniquely, transduction of TVA+ progenitors with a retrovirus encoding a puromycin resistance gene allows selection and expansion of a multipotential hematopoietic progenitor population that can be superinfected with high efficiency. Using this system we show for the first time that v-Myb oncoproteins expressed from avian viruses can induce a leukemic transformation in the mouse. The phenotype of the transformed cells is similar to that which is seen in the chicken and is likewise dependent on the particular structure of v-Myb. This implies that the basic mechanisms of action of mutated transcription factors in the etiology of leukemia are conserved between birds and mammals.

Targeting hematopoietic progenitors is of great interest from the point of view of fundamental experimentation into events that regulate their commitment and differentiation and as a means to model the events leading to the initiation and progression of some types of leukemia and a variety of myeloproliferative diseases. Retroviral and lentiviral vectors are commonly used to introduce genetic material into cells of interest (1). Directing infection toward specific cells has been attempted by targeting cell surface proteins that are expressed on a restricted subset of cells, but the successes have been limited (2, 3). Attempts to target retroviral infection to hematopoietic progenitors have exploited the presence of SCF1 receptor (c-Kit) and CD34 on their surface (4, 5) or have utilized a modified retroviral envelope; however, only very low efficiencies could be obtained compared with conventional retroviral vectors (6, 7).

Avian retroviruses, unlike their mammalian counterparts, cannot replicate in mammalian cells, because of the lack of a cell surface receptor and incomplete intracellular virus assembly (8). However, engineered expression of the subgroup A avian leukemia virus (ALV-A) receptor, TVA, confers susceptibility to infection by ALV-A viruses and by murine retroviruses pseudotyped with the ALV-A envelope protein (9, 10). By expressing TVA from tissue-specific promoters, it has been possible, for example, to restrict retroviral infection to muscle cells (11), glial cells (12), and megakaryocyte-lineage cells (13).

Recent identification of a hematopoietic progenitor-specific enhancer from the scl (tal1) gene offers the opportunity to achieve the desired pattern of expression of TVA. The scl gene encodes a basic helix-loop-helix transcription factor that is essential for the normal development of the blood and blood vessels (reviewed in Ref. 14). It is expressed in the hematopoietic stem cell and possibly earlier before commitment to cells with restricted hemogenic potential. An enhancer element located 3' of the scl gene can direct reporter gene expression to the majority of early hematopoietic progenitors in transgenic mice (15). Long term re-population assays have shown that the scl enhancer can direct transgene expression to early myeloid and erythroid progenitors and to the vast majority of hematopoietic stem cells in adult bone marrow (16).

Here we describe the use of the scl 3' enhancer to direct expression of a tva transgene to the hematopoietic stem cell and progenitor compartment in adult bone marrow and during embryonic development. TVA expression in these tissues is shown to be a specific and efficient means to permit introduction into multipotential hematopoietic progenitors of exogenous coding sequences.

Leukemic transformation is frequently associated with the expression of transcription factors that have acquired distinct regulatory properties as the result of mutation, often brought about by fusion to sequences by specific chromosomal rearrangements. The consequences of the perturbed transcription factor function leading to leukemia are thought to operate in hematopoietic progenitors and precursors. Attempts to model

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†† The abbreviations used are: SCF, stem cell factor; ALV, avian leukemia virus; E, embryonic day; PE, phosphatidylethanolamine; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorter; GFP, green fluorescent protein; EGFP, enhanced GFP; PURO, puromycin; AMV, avian myeloblastosis virus; TPO, thrombopoietin; II, interleukin; FL, FLT-3 ligand; 5-FU, 5-fluouracil.
the initiation and progression of leukemic development have met with varied success, a major difficulty being the specificity and efficiency of introduction of the mutated transcription factors into relatively rare and inaccessible progenitors in the sites of hematopoiesis. Here we show that infection through the TVA receptor in cells from scl-tva mice can be used to assess the transformation potential of oncogenic transcription factors. Our results with v-Myb proteins encoded by avian acute leukemia viruses suggest that this approach is effective ex vivo, and the wider potential for in vivo application is discussed. Interestingly, the phenotype of transformation achieved using murine cells very closely resembled that seen using chicken hematopoietic cells suggesting that basic mechanisms of leukemia involving transcriptional regulators are conserved.

EXPERIMENTAL PROCEDURES

Generation of Transgenic Mice—The cDNA encoding the 950-bp TVA transcript was obtained as a 1.0-kbp HindIII-EcoRI fragment from pGPIb-tva and ligated upstream of the murine scl 3' enhancer element (a 5.5-kbp BgII fragment of the mouse scl locus (15)) and downstream of the murine scl promoter fragment (a 2.8-kbp of mouse scl exon 4) to generate p6E5/TVA3'E (Fig. 1A). A 9.0-kbp XhoI fragment from p6E5/TVA3'E was injected into CBA/Ca x C57Bl/6 F2 fertilized eggs using standard procedures (17). Distal tail segments from potential founder mice were digested overnight at 55 °C in 100 mM Tris-HCl, 5 mM EDTA, 0.2% SDS, and proteinase K, and DNA was precipitated with ethanol.

Specific Infection of Hematopoietic Progenitors

In vitro bone marrow and fetal liver infections were performed using 5 × 10^6 or greater, especially using RCAS vectors modified by insertion of the Bryan strain pol region (RCASBP; see Ref. 8). Such RCASBP-based vectors were employed that expressed either an enhanced green fluorescent protein (RCAS-EGFP) or the puromycin resistance gene (RCAS-FURO; see Ref. 13). Retroviruses expressing the E26 and AMV v-Myb transforming oncoproteins were generated by transfecting the plasmids pMi3 and pMi4, respectively (19), into 293T cells, together with plasmids encoding Gag-pol and EnvA, as described previously (13).

Culture and Infection of Bone Marrow and Fetal Liver Cells—Freshly harvested femurs and tibias were flushed with Hank's-buffered salt solution containing 1% bovine serum albumin. E12 fetal livers were dissected out, and a single cell suspension was obtained by passage through a 25-gauge needle. Mature red blood cells were depleted by incubation in ACK buffer for 5 min at room temperature. The cells were resuspended in specific growth medium to promote progenitor maintenance and expansion (20, 21). All media contained Iscove's modified Dulbecco's medium supplemented with 10% horse serum (Sigma), 100 units/ml penicillin G, and 0.1 mg/ml streptomycin sulfate. For production of virus, DF1 cells 70% confluent in 10-cm plates were transfected with 10 μg of RCAS vector DNA using the calcium phosphate co-precipitation method. Medium was changed 6–8 h after exposure to the precipitate and the day following transfection. 5 days post-transfection, greater than 95% of the DF1 cells showed expression of the exogenous gene, and viral supernatant was harvested and passed through a 0.45-μm syringe filter. The RCAS system typically produces viral titers of 5 × 10^8 or greater, especially using RCAS vectors modified by insertion of the Bryan strain pol region (RCASBP; see Ref. 8). Such RCASBP-based vectors were employed that expressed either an enhanced green fluorescent protein (RCAS-EGFP) or the puromycin resistance gene (RCAS-FURO; see Ref. 13). Retroviruses expressing the E26 and AMV v-Myb transforming oncoproteins were generated by transfecting the plasmids pMi3 and pMi4, respectively (19), into 293T cells, together with plasmids encoding Gag-pol and EnvA, as described previously (13).

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 Colony Assay—Infected bone marrow or fetal liver cells were plated in 2.5 ml of 1% methylcellulose medium (Methocult M3434; Stem Cell
Technologies, Vancouver, British Columbia) containing Iscove’s modified Dulbecco’s medium, 15% fetal calf serum, 0.1 m M 2-mercaptoethanol, 2 m M l-glutamine, 1% bovine serum albumin, insulin (10 mg/ml), iron-saturated human transferrin (200 mg/ml), IL-3 (10 ng/ml), IL-6 (10 ng/ml), SCF (50 ng/ml), Epo (3 units/ml), and TPO (25 ng/ml). Triplicate cultures were incubated for 7 days at 37°C in a fully humidified 5% CO2 atmosphere, and colony numbers were assessed on day 3 (CFU-E), day 6 (BFU-E), and day 10–12 (CFU-mixed and myeloid colonies) using both light and fluorescent microscopes.

**CFU-S12 Assay**—The spleen colony assay of Till and McCulloch (22) was used to assay for the stable infection of CFU-S (colony-forming unit-spleen) multipotent progenitors. E12 scl-tva fetal liver cells were cultured for 3 days in Iscove’s modified Dulbecco’s medium containing 10% horse serum, penicillin/streptomycin, FL (10 ng/ml), SCF (20 ng/ml), IL-6 (10 ng/ml), SCF (50 ng/ml), Epo (3 units/ml), and TPO (25 ng/ml) and were infected with filtered supernatant from RCAS-EGFP-infected DF1 producer cells. C57BL/6 female recipients 8 to 12 weeks of age were exposed on the day of transplantation to a single dose of 10.0 gray of radiation from dual opposed Co60 sources at an exposure rate of 0.2 gray/min. 5 × 10⁵ infected cells were injected into irradiated mice. The mice were sacrificed 12 days later, and their spleens were dissected, fixed in Bouin’s solution for 5 min, then transferred to 10% neutral buffered formalin. Prior to fixation, GFP-positive colonies were visualized using an inverted fluorescence microscope at low power. Quantification of the total number of colonies was performed after fixation, which renders them more visible under normal light.

**In Vivo Infections**—6- to 8-week-old scl-tva mice and non-transgenic controls were primed by subcutaneous injection of 150 mg/kg 5-fluorouracil (5-FU) and injected intravenously via the tail vein with 1 × 10⁶ RCAS-EGFP DF1 producer cells on day 4 post-5-FU administration. 10 weeks and 6 months after injection of the virus-producing cells, the mice were sacrificed, and bone marrow was harvested. Single cell suspensions were treated with ACK buffer to lyse red blood cells prior to immunofluorescence and flow cytometric analysis.

### RESULTS

**The scl 3’ Enhancer Targets TVA Expression to the Progenitor Cell Compartment**—To analyze TVA expression in hematopoietic progenitors from scl-tva transgenic mice, we prepared bone marrow from adults at 6–8 weeks of age and from the fetal livers of E12 embryos. Cell staining using an anti-TVA rabbit antiserum demonstrated TVA expression on 14.7% of...
red cell-depleted bone marrow cells (Fig. 2A) and 16.0% of red cell-depleted fetal liver cells (Fig. 2C). Simultaneous staining for markers characteristic of immature hematopoietic cells showed that virtually all of the CD34⁺ cells in both bone marrow and fetal liver were also TVA⁺ (Fig. 2, A and C). In Fig. 2B additional stainings of bone marrow are represented to show that TVA is expressed on cells with characteristics of progenitor cells but not on cells expressing terminal differentiation antigens. Hence, gating on cells positive for the individual antigens showed that −66% of the c-Kit⁺ population is TVA⁺ whereas cells expressing low levels of the Mac1 antigen were 44% positive for TVA. Conversely, few, if any, cells expressing high levels of Mac1 or positive for the erythroid marker Ter119, the megakaryocyte marker CD41, or the B cell marker B220 were simultaneously positive for TVA.

To further define the nature of the CD34⁺ TVA⁺ population and what the CD34⁺ TVA⁺ cells represent we performed three-color staining of fetal liver cells for TVA, CD34, and c-Kit. This revealed that 92% of c-Kit⁻ CD34⁺ cells were TVA⁺ (Fig. 2D, population 3) whereas CD34⁺ cells that expressed low levels of c-Kit were 95% TVA⁺ (Fig. 2D, population 4). TVA⁺ cells that were CD34⁻ appeared to be mainly c-Kit⁻ (Fig. 2D, population 2). The three-color staining also demonstrated that cells expressing low levels of c-Kit and low levels of CD34 were mostly TVA⁻ (Fig. 2D, population 1).

Next, using immunomagnetic bead depletion and high speed sorting of immunofluorescently labeled cells, we enriched a population that contained hematopoietic stem cells with the surface antigen phenotype Lin⁻ ’Sca¹’c-Kit⁺ (23). These sorted cells were positive for staining using the anti-TVA antibody (Fig. 2E). This pattern of TVA expression was therefore consistent with that reported previously (15) for the slc 3’ enhancer as detected by use of a lacZ reporter and implies that TVA is present on hematopoietic stem cells, progenitors, and precursor cells.

Hematopoietic Cells from slc-tva Mice Can Be Infected with Avian Retroviruses—Next we infected fetal liver cells from E12 slc-tva embryos using RCAS-EGFP in the presence of FL, IL-6, SCF, and TPO to maintain the progenitor cell compartment in vitro (20, 21). Flow cytometric analysis of the infected population after 2 days in culture showed that −7.0% of the cells expressed detectable levels of GFP as compared with the control cells, which were obtained by RCAS-EGFP infection of non-TVA-expressing fetal liver (Fig. 3, A and B). Simultaneous immunofluorescent staining of the infected cells indicated that the GFP-expressing cells were predominantly positive for the progenitor markers CD34, c-Kit, and, to a lesser extent, Sca-1 (Fig. 3, C–E) but that only a small proportion exhibited positivity for the erythroid marker Ter119 (Fig. 3F). These data demonstrate that this system can be used to target retroviral infection to a subset of hematopoietic cells that express the markers of hematopoietic progenitors and precursors.

Progenitor Activity of slc-tva Fetal Liver Cells Infected with RCAS-EGFP— Colony assays were performed to define functionally the nature of hematopoietic progenitor and precursor cells that could be infected through the TVA receptor. Fetal liver cells were harvested from E12 embryos, cultured in 10 ng/ml FL, 20 ng/ml SCF, 25 ng/ml TPO, and 10 ng/ml IL-6 and infected with RCAS-EGFP for two successive days followed by seeding in methylcellulose for determination of specific CFU content. Although three distinct types of GFP-positive colonies were noted based on their morphology (mixed, pure myeloid, and pure erythroid), the ratio of GFP⁺ to GFP⁻ mixed colonies (2.0) was much greater than that seen for pure myeloid (0.7) or pure erythroid colonies (0.15) (Fig. 4A). These results indicate that the majority of the multipotent progenitors but only a minority of the unilineage progenitors had been infected. We cannot, however, determine whether GFP expression in lineage-committed cells is the result of direct, albeit less efficient, infection of monopotent precursors or of post-infection commitment of infected multipotent progenitors before seeding into semi-solid medium.

The CFU-S assay as first described by Till and McCulloch (22) provides one means to selectively quantify multipotent progenitors present in a population of cells. Such assays were therefore performed to determine what proportion of multipotent progenitors present in an ex vivo culture could be infected via the TVA receptor. Lethally irradiated mice were transplanted with 5 × 10⁴ slc-tva fetal liver cells that had been infected with RCAS-EGFP virus and cultured for 2 days in the growth factor mixture described above. The percentage of fetal liver cells injected into the mice that expressed GFP was similar to that described for the in vitro RCAS-EGFP infections in Fig. 3B (7.0%). Examination of the spleens of the transplanted mice after 12 days revealed an average of 6.3 colonies per spleen (Fig. 4, B and C), with 68.5% of the colonies expressing GFP (Fig. 4C). These data demonstrate that the majority of CFU-S₁₂₁ progenitors which are maintained in a multipotential state during culture and infection ex vivo can be infected with RCAS-EGFP using this technique.

Production of an Enriched Population of Early Hematopoietic Progenitors from slc-tva Bone Marrow— Bone marrow from sel-
tva mice was cultured in FL, SCF, TPO, and IL-6 and infected with RCAS-PURO. These growth conditions maintain the early compartment of hematopoietic progenitors while still allowing distinct lineage differentiation to take place (20, 21). Following selection for 7 days in puromycin (1 µg/ml), a population of cells emerged that showed the hallmarks of immature hematopoietic progenitors. The majority of cells expressed the progenitor markers CD34 and Sca1 (64.1 and 58.8%, respectively), whereas 14.6% expressed c-Kit (Fig. 5A). Conversely, almost none of the selected cells expressed Ter119, a marker of the erythroid lineage (Fig. 5A). May-Grünwald staining of the selected population showed a mixed population of blast-like cells (Fig. 5B). Methylcellulose colony assays revealed that an average of 71% of the puromycin-selected cells formed colonies (data not shown).

In mammalian cells the viral env gene is expressed at rela-
tively low levels so that TVA-expressing cells are susceptible to superinfection by additional avian retroviruses. To test the feasibility of multiple sequential infection of hematopoietic progenitors derived from scl-tva bone marrow, cells infected with RCAS-PURO were selected in puromycin for 7 days and then infected with RCAS-EGFP. During the same 7-day period, non-TVA-expressing control bone marrow cells infected with RCAS-PURO and grown in the presence of puromycin all died (data not shown). FACS analysis of the superinfected cells demonstrated that 79% of the puromycin-resistant cells express GFP at 2 days post-RCAS-EGFP infection (Fig. 5C). Together, these data indicate that the scl-tva mice can be used to produce a highly enriched population of early hematopoietic cells that can then be further genetically manipulated by retroviral superinfection.

**Targeting Potential Oncoproteins to Hematopoietic Progenitors**—As a further illustration of the feasibility of using the scl-tva transgene as a means to target hematopoietic progenitors we infected bone marrow with avian leukemia viruses that have been shown previously to transform progenitor or precursor cells in the chicken (19, 24). Specifically, we made use of the E26 virus encoding the 135-kDa Gag-myb-ets fusion oncoprotein (25, 26), which has been shown to transform both multipotent myeloid and bipotential myelomonocytic progenitors (24), and the AMV virus encoding the 45-kDa truncated c-Myb protein, which transforms monoblastic cells (19). E26 and AMV viruses produced from the DF1 packaging line were used to infect 5 × 10⁵ scl-tva bone marrow cells for 2 days in the presence of FL, SCF, TPO, and IL-6. Control infections were performed using an empty retroviral vector. On day 3 the cells were placed in medium containing 15% fetal calf serum and no added growth factors. Monitoring of cell numbers showed a clear outgrowth of transformed cells from the virus-infected cultures (Fig. 6A). At day 12 the E26- and AMV-transformed cells had the characteristic morphology of immature blasts and monoblasts, respectively (Fig. 6B). These phenotypes were reflected in the expression of surface markers (Fig. 6C). Hence, the E26-transformed cells immature cells were positive for CD34 and c-Kit (Fig. 6C), whereas the AMV-transformed monoblasts expressed the marker Mac1 but were negative for CD34 and c-Kit expression. During continued culture, the progenitor phenotype of the E26-transformed cells was progressively lost as cells differentiated toward adherent, Mac1⁺ monocyte-macrophage cells (data not shown).

**Targeted Retroviral Infection of Hematopoietic Progenitor Cells in Vivo**—In a preliminary attempt to demonstrate the
feasibility of targeted infection of progenitor cells in vivo, 6- to 8-week-old scl-tva mice and non-transgenic controls were primed with 5-FU and injected 4 days later with 1 x 10^6 RCAS-EGFP DF1 producer cells. 10 weeks or 6 months later the mice were sacrificed, and bone marrow and spleens were harvested and treated to lyse red cells prior to immunofluorescence analysis. A, merged fluorescent and transmission images of spleen cells from an RCAS-EGFP-infected scl-tva transgenic mouse. B, flow cytometric analysis of 5 x 10^6 bone marrow cells from an RCAS-EGFP-infected scl-tva transgenic mouse 10 weeks after injection of the virus-producing cells. C, two-color immunofluorescence analysis of bone marrow cells from an RCAS-EGFP-infected scl-tva transgenic mouse six months after injection of the virus-producing cells. The extent of green fluorescence is indicated on the horizontal axis whereas the vertical axis indicates streptavidin-PE binding to control antibody or specific antibodies against CD34, c-Kit, Sca-1, Ter119, and Mac1 as indicated.

**DISCUSSION**

TVA-based retroviral transduction has been developed as a means to introduce exogenous genetic material selectively and efficiently into somatic cells both ex vivo and in vivo. There are several advantages of infection through the TVA receptor using an ALV-based retroviral vector system in mammalian cells. Specificity of infection is determined solely by the expression domain of the TVA-expressing transgene. Moreover, highly efficient infection is facilitated, because ALV vectors can be quickly and easily grown to high titers as replication-competent viruses on avian cells. The fact that the viral env gene is inefficiently expressed in mammalian cells means that superinfection is not precluded as would be the case using conventional murine retroviral systems. This, therefore, allows for the expression of more than one gene in a given cell by multiple sequential infections.

Until now, the only application of TVA technology within the hematopoietic system has been for the infection of cells committed to the megakaryocyte lineage (13). Here, we have generated and characterized a new TVA transgenic mouse strain, scl-tva, which makes it possible to infect hematopoietic cells prior to commitment to terminal differentiation. Our results demonstrate that the scl 3’ enhancer can direct tva transgene expression to the hematopoietic progenitor compartment in a manner very similar to that seen for lacZ in the original characterization of this enhancer (15). The fact that most, if not all, CD34+ cells also express the tva transgene implies that a large part of the progenitor population can potentially be targeted by retroviral infection. Moreover, the presence of TVA on Lin Sca1-c-Kit+ cells indicates that hematopoietic stem cells could also be infected. Using the scl-tva mouse we have shown that avian retroviruses carrying a gene product of choice can selectively infect hematopoietic progenitors and precursors. Among the TVA+ population are multipotential cells, which, based on the CFU-S assay, can be infected ex vivo at high efficiency (~70%) and still maintain their capacity as functional progenitors when transplanted in vivo. Indeed, this is the best indication of the efficiency of infection of progenitors given that it was not possible to assess the absolute level of infection by RCAS-EGFP, because during culture of the infected population many of the initially TVA+ cells would have become TVA-+, and those cells seen as being GFP+ at the time of analysis were hence the result of a complex dynamic of self-renewal, differentiation, and cell death.

The scl-tva mouse will certainly be useful for studying the
influence of transcription factors or other proteins on hematopoietic lineage choice. By permitting infection and expression of a particular factor prior to lineage commitment, it should be possible to influence lineage choice and differentiation *ex vivo*. In this context, it is particularly interesting that by infection of *scl-tva* hematopoietic cells with RCAS-PUR0 followed by anti-biotic selection we were able to produce an enriched population of primary multipotent progenitors that were highly susceptible to superinfection by a second retrovirus. We are currently investigating these cells in greater detail with regard to their phenotype and potential to undergo effective multilineage differentiation.

We have shown that infection of murine hematopoietic progenitors with the viruses E26 and AMV expressing oncogenically activated derivatives of the transcription factor c-Myc results in the outgrowth of homogenous populations of cells. Interestingly, although E26 and AMV are avian leukemogenic viruses, the phenotypes of the infected cell populations derived from mouse bone marrow resembled those obtained when the viruses transform chicken hematopoietic progenitors (19, 24). This degree of similarity between the effect of transforming Myb proteins in mouse and chicken is particularly interesting with respect to the E26 Gag-myb-ets protein. The immature progenitor phenotype of the expanded population infected with E26 appears to be analogous to the multipotent progenitor transformed from chicken hematopoietic cells (24), although our culture conditions seemed to favor eventual commitment to monocytic differentiation. In contrast, an earlier alternative approach at examining the effect of Gag-myb-ets on murine hematopoietic cells involving insertion of the oncoprotein coding sequences between murine retroviral long terminal repeats of hematopoietic cells involving insertion of the oncoprotein coding sequences between murine retroviral long terminal repeats gave rise to transformed erythroid cells (27).

From the viewpoint of the manipulation of lineage choice of multipotent progenitors the *scl-tva* system also has the potential to be extended through use of viruses derived from the RCAN retroviral backbone. These viral vectors lack a splice acceptor and allow for the use of a specific internal promoter to drive exogenous gene expression (8). Other investigators (28) have shown that a retrovirus containing an erythroid-specific promoter can be used to transfer genes into the erythroid lineage with resultant high level gene expression. However, these studies have been conducted only in cells already committed to the erythroid lineage. Using an RCAN-based virus capable of expressing EGFP from an internal erythroid promoter/enhancer we have preliminary results that suggest that cells derived from the *scl-tva* mouse can be infected at an immature stage that already express a high level of exogenous gene expression but are restricted to the red cell compartment (data not shown).

Simple retroviruses fail to integrate in non-replicating target cells because of a block that occurs before entry into the nucleus of the infected cell (29). This is a problem relevant to the targeting of hematopoietic stem cells, because they are mostly quiescent (30). In this context, we have recently been able to pseudotype a feline immunodeficiency virus-based lentiviral vector with subgroup A avian leukaemis virus envelope protein.2 This should allow the use of a lentiviral vector capable of infecting non-dividing cells while still utilizing the targeting system enabled by the specific expression of TVA.

In *vivo* gene transfer into hematopoietic cells is extremely enticing, but in practice this has proven to be difficult (31). Previous observations based on purification of bone marrow cells expressing the lacZ transgene driven by the *scl* promoter/enhancer (16) strongly suggest that it should be possible to target hematopoietic stem cells using the *scl-tva* mouse. Although the efficiency was low, our initial attempts at targeted *in vivo* infection have shown that this appears to be possible in that we obtained infected cells that persisted long term and had the characteristics of hematopoietic progenitors.

Even with a low efficiency of infection of stem cells or multilineage progenitors in *vivo* the *scl-tva* mouse has potential utility in the generation of models of hematological malignancies in which it is anticipated that the transduced gene will confer a considerable proliferative advantage. Such an approach would also make it possible to investigate the influence of genetic background on the activity of the gene of interest. We are presently investigating the potential of this approach with a number of fusion proteins associated with specific myeloid leukemias, and in this context our results with the avian v-myc encoding leukemic retroviruses are very encouraging.

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**REFERENCES**

1. Miller, A. D. (ed.) (1997) *Development and Applications of Retroviral Vectors*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
2. Lin, A. H., Kasaqara, N., Wu, W., Stripkeo, B., Empi, C. L., Anderson, W. F., and Cannon, P. M. (2001) *Hum. Gene Ther.* 12, 323–332.
3. Paarss, K., Ueda, M., Tanabe, M., Kitajima, A., Iso, S., Komatsu, S., and Seno, M. (2000) *Cytokine* 12, 786–790.
4. Schwaarzenberger, P., Spence, S. F., Goya, J. M., Michel, D., Curie, D., Rusteci, F. W., and Keller, J. R. (1996) *Blood* 87, 472–478.
5. Yajima, T., Kanda, T., Yoshikai, K., and Kitamura, Y. (1998) *Hum. Gene Ther.* 9, 779–787.
6. Cosset, F. L., Morling, F. J., Takeuchi, X., Weiss, R. A., Collins, M. K., and Russell, S. J. (1995) *J. Virol.* 69, 6314–6322.
7. Kasaqara, N., Duy, A. M., and Kan, Y. W. (1994) *Science* 266, 1373–1376.
8. Federspiel, M. J., and Hughes, S. H. (1997) *Methods Cell Biol.* 52, 179–214.
9. Bates, P., Young, J. A., and Varum, H. E. (1991) *Cell* 74, 1043–1051.
10. Young, J. A., Bates, P., and Varum, H. E. (1993) *J. Virol.* 67, 1811–1816.
11. Federspiel, M. J., Bates, P., Young, J. A., Varum, H. E., and Hughes, S. H. (1994) *Proc. Natl. Acad. Sci. U. S. A.* 91, 11241–11245.
12. Holland, E. C., and Varum, H. E. (1998) *Proc. Natl. Acad. Sci. U. S. A.* 95, 8218–8223.
13. Murphy, G. J., and Leavitt, A. D. (1999) *Proc. Natl. Acad. Sci. U. S. A.* 96, 3065–3070.
14. Begley, C. G., and Green, A. B. (1999) *Blood* 93, 2760–2770.
15. Sanchez, M. J., Gittens, B., Sinclair, A. M., Stanley, M., Begley, C. G., Hunter, S., and Green, A. B. (1999) *Development* 126, 3891–3904.
16. Sanchez, M. J., Beckamp, E. O., Miller, J., Gambardella, L., and Green, A. R. (2001) *Development* 128, 4815–4827.
17. Hogan, B., Costantini, F., and Lacy, E. (1986) *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
18. Coligan, J. E., Krujivek, A. M., Margulies, D. H., Shevach, E. M., and Strober, W. (eds) (1995) *Current Protocols in Immunology*, John Wiley & Sons, Inc., New York.
19. Introna, M., Gelay, J., Frampton, J., Nakano, T., Ness, S. A., and Graf, T. (1990) *Cell* 63, 1289–1297.
20. Kobari, L., Giarritana, M. C., Poloni, A., Firtat, H., Labopin, M., Gorin, N. C., and Douay, L. (1998) *Bone Marrow Transplant.* 8, 759–767.
21. Velhey, O. P., Jacobsen, W. F., Cui, L., Lyman, S. D., and Jacobsen, S. E. (1996) *J. Immunol.* 157, 2963–2969.
22. Till, J. E., and McCulloch, E. A. (1961) *Radiat. Res.* 14, 213.
23. Spangrude, G. J., Brooks, D. M., and Turnans, D. B. (1995) *Blood* 85, 1006–1016.
24. Graf, T., McNagny, K. M., Brady, G., and Frampton, J. (1992) *Cell* 70, 201–213.
25. Mosenm, M. G., Jurdie, P., Samarut, J., Gamba, L., Mura, C., and Moscovici, C. (1983) *Virology* 129, 65–78.
26. Leprince, D., Gogonea, A., Coll, J., deTaisne, C., Schneebeger, A., Lagrou, C., and Stehelin, D. (1988) *Nature* 336, 385–387.
27. Rusteci, S., Argirimera, R., Yuan, C. C., Sawyer, S., and Blair, D. G. (1992) *J. Virol.* 66, 20–26.
28. Stein, C., Kang, Y., Saeter, S., Townsend, K., Staber, P., Derksen, T., Martin, I., Qian, J., Davidson, B., and McCray, P. (2001) *Mol. Ther.* 3, 850–856.
29. Miller, D. G., Adam, M. A., and Miller, A. D. (1990) *Mol. Cell Biol.* 10, 4329–4342.
30. Hodgson, G. S., and Bradley, T. R. (1979) *Nature* 281, 381–382.
31. Heim, D. A., and Danhar, C. E. (2000) *Immunol. Rev.* 178, 29–38.

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2 G. J. Murphy, A. D. Leavitt, and J. Frampton, unpublished observations.