Human Cord Blood Cells as Targets for Gene Transfer: Potential Use in Genetic Therapies of Severe Combined Immunodeficiency Disease

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Summary

Human cord blood (CB) contains large numbers of both committed and primitive hematopoietic progenitor cells and has been shown to have the capacity to reconstitute the lympho-hematopoietic system in transplant protocols. To investigate the potential usefulness of CB stem and progenitor cell populations to deliver new genetic material into the blood and immune systems, we have transduced these cells using retroviral technology and compared the efficiency of gene transfer into CB cells with normal adult human bone marrow cells using a variety of infection protocols. Using two retroviral vectors which differ significantly in both recombinant viral titers and vector design, low density CB or adult bone marrow (ABM) cells were infected, and committed progenitor and more primitive hematopoietic cells were analyzed for gene expression by G418 drug resistance (G418^r) of neophosphotransferase and protein analysis for murine adenosine deaminase (mADA). Standard methylcellulose progenitor assays were used to quantitate transduction efficiency of committed progenitor cells, and the long term culture-initiating cell (LTC-IC) assay was used to quantitate transduction efficiency of more primitive cells. Our results indicate that CB cells were more efficiently transduced via retroviral-mediated gene transfer as compared with ABM-derived cells. In addition, stable expression of the introduced gene sequences, including the ADA cDNA, was demonstrated in the progeny of infected LTC-ICs after 5 wk in long-term marrow cultures. Expression of the introduced ADA cDNA was higher than the endogenous human ADA gene in the LTC-IC–derived colonies examined. These studies demonstrate that CB progenitor and stem cells can be efficiently infected using retroviral vectors and suggest that CB cells may provide a suitable target population in gene transfer protocols for some genetic diseases.

Recent advances in the understanding of human diseases at the molecular level has led to the routine use of prenatal diagnosis in several severe genetic conditions. In addition, the increasingly successful application of gene transfer technology, particularly the use of recombinant retroviral vectors, holds the promise of a lifelong cure of some of these diseases by somatic gene therapy (1, 2). Although reproducible and efficient gene delivery to bone marrow stem cells has been achieved by a number of investigators in murine studies (3–8), the successful extension of this technology to larger animals (canines and primates) has been problematic (9–12). Gene transfer into the most primitive hematopoietic stem cell responsible for long-term reconstitution has been very inefficient in canine and primate studies. In spite of the relatively high transduction efficiency of more differentiated progenitor cells, high-level expression of introduced genetic sequences has not been routinely seen in large animal experiments (10, 11). These limitations of current gene transfer tech-

nology are further complicated when applied to human protocols by the low numbers of stem cells present in adult bone marrow (ABM)[1], lack of suitable methods to purify these cells, and the high fraction of such primitive cells that are not in cell cycle (for a review see reference 13).

Human cord blood (CB) has previously been demonstrated to contain a large number of primitive progenitor cells (14, 15). Although no direct in vitro assay is available to determine the content of reconstituting hematopoietic stem cells among human cells, multiple investigators have now demonstrated the capacity of single CB collections to reconstitute

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[1] *Abbreviations used in this paper:* ABM, adult bone marrow; mADA, murine adenosine deaminase; BFU-E, burst forming unit-erythroid; BMF, bone marrow fibroblast; CB, cord blood; CDB, cell dissociation buffer; LTC-IC, long term culture-initiating cell; LTMC, long term bone marrow culture; NEO, neophosphotransferase; PGKpr, phosphoglycerate kinase promoter; P/S, penicillin/streptomycin; SCF, stem cell factor.

the lympho-hematopoietic system of infants and children following transplantation in vivo after myeloablative therapy (16, 17). Recent in vitro data suggest that single CB samples may also be sufficient to reconstitute hematopoiesis in adult recipients (18, 19). In addition, analysis of cycling rates of CB CFU-GM cells demonstrate that substantial numbers of these cells are in cell cycle (20, 21).

To determine if CB cells might be successfully transduced using retroviral vectors, we compared infection efficiencies of CB progenitor and stem cells with that of normal ABM using the neophosphotransferase (NEO) gene in identical vectors and infection protocols. In addition, expression of a clinically relevant gene sequence, adenosine deaminase (ADA) cDNA, the deficiency of which causes SCID, was examined. We report that committed and primitive hematopoietic cells from human CB are efficiently infected using retroviral vectors, and that the introduced sequences are expressed at high levels in progeny of transduced stem cells.

Materials and Methods

Retroviral Vectors, Producer Cell Lines, and Stromal Cells. The N$_2$/ZipTKNEO (TKNEO) vector is identical to the previously reported N$_2$/ZipPGK-ADATKNEO (22) vector except the phosphoglycerate kinase promoter (PGKpr)-hADA expression cassette has been removed (see Fig. 1 *A*). NEO sequences are expressed in the sense orientation (relative to the 5' LTR) via the thymidine kinase promoter. The ZipPGK-mADA (PGK-mADA) vector is identical to the ZipPGK-hADA vector (23) except the human ADA cDNA has been replaced with the murine ADA cDNA (see Fig. 1 *B*). The mADA cDNA is expressed in the sense orientation relative to the 5'LTR via the human PGK promoter. GP+EnvAm 12 (24) producer cells containing either retroviral plasmid (TKNEO or PGKmADA) were cultured in IMDM (GIBCO BRL, Gaithersburg, MD) containing 10% FCS (Hyclone Laboratories, Logan, UT) and 100 U/ml penicillin and 100 μg/ml streptomycin (P/S, both from GIBCO BRL). Virus-containing supernatant was collected by adding 10 ml of IMDM containing 20% FCS to confluent plates overnight. Harvested medium was filtered through 0.45-μm filters (Gelman Sciences, Inc., Ann Arbor, MI) and stored at -80°C until used. For coculture experiments, producer cells were treated with 10 μg/ml mitomycin C (Sigma Chemical Co., St. Louis, MO) for 2 h at 37°C, washed three times with PBS, trypsinized, and plated at confluence on the day before infection.

Stromal Cells. Sl^4-h220 cells (25), a murine-derived and genetically modified stromal cell line that expresses high levels of membrane-bound human Steel factor (or stem cell factor [SCF]) were maintained in DME (GIBCO BRL) supplemented with 10% calf serum (CS, Sigma Chemical Co.), and P/S. The day before use in the infection protocol, the cells were treated with 5 μg/ml mitomycin C for 2 h, prepared as above, and plated at confluence on tissue culture plates (Corning Inc., Corning, NY) precoated with 0.1% gelatin (Sigma Chemical Co.). Human allogeneic bone marrow fibroblasts (BMF) were grown in DME supplemented with 10% FCS and P/S. BMF were irradiated at confluence with 15 Gy using a gamma cell 40 cesium source.

Retroviral Infection Protocol. CB samples from normal, full-term, newborn infants and bone marrow samples from healthy adult donors were collected in tubes containing heparin according to protocols approved by the Institutional Review Board of Indiana University School of Medicine. Low density mononuclear cells from each

sample were prepared by centrifugation on Ficoll-Hypaque (density 1.077 g/ml; Pharmacia, Piscataway, NJ) for 45 min at 25°C. Plastic adherent cells were removed from low density bone marrow cells by an additional incubation on tissue culture plates for 4-16 h at 37°C in 5% CO$_2$ in IMDM with 10% FCS. Adherent-negative low density mononuclear cells and low density CB cells were prestimulated before retroviral infection as described previously (5) for 48 h at 37°C and 5% CO$_2$ in IMDM containing 20% FCS, 100 U/ml rhIL-6, 100 ng/ml rhSCF (both from Amgen Biologicals, Thousand Oaks, CA), and P/S at a cell density of 10^6 cells/ml in petri dishes (Falcon, Lincoln Park, NJ). Prestimulated cells were harvested by vigorous pipetting (to remove cells loosely adherent to the plastic) and then exposed to virus using four different infection protocols: (*a*) coculture: prestimulated cells were overlaid on mitomycin C-treated (see above) retroviral producer lines (GP+envAm 12-TKNEO or GP+envAm 12-PGK-mADA); (*b*) supernatant infection on genetically modified stromal cells: prestimulated cells were plated and infected on mitomycin C-treated Sl^4-h220 cells; (*c*) supernatant infection on allogeneic BMF: prestimulated cells were plated and infected on confluent and irradiated BMF; and (*d*) supernatant infection: prestimulated cells were exposed to virus in tissue culture plates without producer or stromal cells present. All infections were performed on 10-cm tissue culture plates in 10 ml of media with 5 × 10^5/ml prestimulated cells and 5 μg/ml polybrene (Aldrich Chemical Co., Milwaukee, WI), rhIL-6, rhSCF (concentrations as above) for 48 h. For supernatant protocols, 10 ml of virus-containing medium supplemented with fresh growth factors and polybrene was replaced every 12 h for a total of four medium changes (nonadherent cells were re-added with each medium change). After the infection protocol, nonadherent and adherent hematopoietic cells were collected from the cultures using cell dissociation buffer (CDB) (enzyme free/PBS based; GIBCO BRL) according to the manufacturer's instructions, washed twice, and counted. These cells were either plated in clonogenic methylcellulose assays or long-term bone marrow cultures (LTMC) (see below). In some experiments, an additional incubation on tissue culture plates to remove plastic adherent cells was performed after the infection protocol.

Bone Marrow Cultures. LTC-IC assays were performed according to previously described methods (26) with modifications. Briefly, 0.5-1.0 × 10^6 infected cells were seeded in LTMC on confluent, preirradiated (as above) allogeneic human BMF in 5 ml IMDM containing 10% FCS, 10% horse serum (Sigma Chemical Co.), P/S, 1 μM hydrocortisone (Upjohn, Kalamazoo, MI), and 320 milliosmole sodium chloride in 6-well tissue culture plates (Costar Corp., Cambridge, MA). LTMC were incubated at 33°C in 5% CO$_2$ and fed weekly by removal of 50% of the media and nonadherent cells. After 5 wk in culture, LTC-IC cultures were killed by using CDB to dissociate adherent hematopoietic cells from BMFs. Both nonadherent and adherent hematopoietic cells were combined and plated in methylcellulose to obtain colonies derived from LTC-IC. In addition, nonadherent cells from long-term cultures were harvested and plated at week 3 of culture to determine the efficiency of gene transfer into less primitive colony-forming cells. In some experiments, a 4-8 h incubation period on tissue culture plates was used to remove remaining plastic adherent cells before initiating methylcellulose assays.

Clonogenic methylcellulose assays were performed as previously described (25) with minor modifications. Briefly, 1-2 × 10^4 infected CB cells or 5 × 10^4 infected ABM cells were plated with 5 U/ml erythropoietin (Epo; Amgen Biologicals), 100 ng/ml rhSCF, 10 ng/ml rhIL-3 (Genzyme Corp., Cambridge, MA) in 1 ml of IMDM methylcellulose (Fluka Chemical Corp., Ronkonkoma, NY)

containing 25% FCS, 10% human plasma, 10^{-5} M β-ME (Sigma Chemical Co.), and P/S. Cultures were incubated at 37°C in 5% CO_2/95% air, and colonies (>50 cells) were scored by viewing on an inverted microscope on day 13 as CFU-GM (containing granulocytes and macrophages), CFU-Mix (containing myeloid and erythroid elements), or burst forming unit–erythroid (BFU-E) (containing only erythroid elements).

Analysis of Retroviral Infection. Efficiency of infection using the TKNEO virus was analyzed by determining the percentage of methylcellulose colonies resistant to G418. Infected hematopoietic cells were plated in the presence or absence of 1.5 mg/ml (dry powder) G418 (GIBCO BRL) and surviving colonies (>50 cells) scored on day 13. Control cultures were performed with each experiment by incubating bone marrow on the GP+EnvAM 12 packaging line (24) making no recombinant virus. Culture of these mock infected cells with 1.5 mg/ml G418 consistently demonstrated <1% background colonies. Efficiency of infection using the PGK-mADA vector was determined by protein analysis using ADA isoenzyme electrophoresis in individual progenitor colonies as previously described (23, 27).

Cell-cycle Analysis of Hematopoietic Progenitor Cells. Tritiated thymidine suicide studies were performed according to the method described by Byron (28). Aliquots of 10×10^6 low density mononuclear cells were incubated in IMDM/20% FCS either containing "cold" (nonradioactive) thymidine, or 0.1 mCi/ml high sp act ($\sim$80 Ci/mM) [methyl-^{3}H]thymidine (New England Nuclear, Boston, MA). After a 20-min incubation, with agitation every 5 min, the reaction was stopped by adding ice-cold IMDM/20% FCS with excess cold thymidine (100 μg/ml). Cells were washed three times and placed into clonogenic methylcellulose assays as described above.

Statistical Analysis. Student's t test was used to compare gene transfer efficiency between CB and ABM samples and between Mix/BFU-E and CFU-GM progenitors. P values of <0.05 and <0.01 were considered to show significant differences in infection efficiency between these groups.

Results

Infection of Committed Progenitor Cells with TKNEO Vector. Gene transfer efficiency into CB-derived committed progenitor cells was initially analyzed using the TKNEO virus (Fig. 1 A) which has a titer of 10^5 G418-resistant (G418^r) CFU/ml on NIH/3T3 cells. Six individual CB samples were analyzed for the generation of G418-resistant progenitor colonies and compared with six ABM samples obtained from healthy adults.

After the infection protocol (which included 4 d of in vitro exposure to SCF and IL-6 in each protocol) most CB-derived red colonies observed in methylcellulose had a large, spread-out morphology and contained >1,000 cells with obvious focal areas containing white cells. Colonies resembling typical burst, derived from BFU-E, were rare and usually contained some white elements when analyzed microscopically after Wright-Giemsa staining, as reported by other investigators (18). Therefore, we included all red colonies into a single CFU-Mix plus BFU-E (Mix/BFU-E) category.

As shown in Fig. 2, gene transfer efficiency into CB cells was consistently two- to threefold higher than into ABM. Coculture of CB yielded an average of 53 ± 12% G418^r Mix/BFU-E colonies compared with 24 ± 11 G418^r colo-
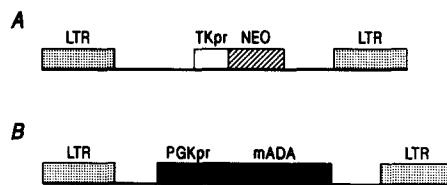
Figure 1. Structure of recombinant retroviral vectors. (*A*) TKNEO virus. (*LTR*) long terminal repeat; (*TKpr*) herpes simplex virus thymidine kinase promoter; (*NEO*) neophosphotransferase sequence. (*B*) PGK mADA virus. (*PGKpr*) Human phosphoglycerate promoter (X-chromosome); (*mADA*) murine adenosine deaminase cDNA.

nies derived from infected ABM. Similarly, coculture of CB yielded an average of 36 ± 17% G418^r CFU-GM colonies compared with 18 ± 7% of progenitors from ABM. For these and all subsequent experiments, we were unable to demonstrate significant differences in the efficiency of infection of Mix/BFU-E and CFU-GM progenitors. Similar differences in the infection efficiencies between CB and ABM committed progenitor cells were noted when these cells were infected without prestimulation (data not shown).

Influence of Infection Protocols on Gene Transfer Efficiency into CB Cells. To determine optimal infection conditions for CB, four different infection protocols were performed. After prestimulation, CB was: (*a*) cocultured with retrovirus producer cells; (*b*) repeatedly infected using supernatant only; (*c*) infected with supernatant while cultured on irradiated allogeneic stromal cells; or (*d*) infected with supernatant while cultured on a genetically modified stromal cell line. Table 1 compares the infection efficiency achieved with the different protocols using the TKNEO vector. In contrast to the high efficiency of gene transfer seen with coculture infection, supernatant infection was generally inefficient. In three of four experiments, supernatant infection generated <5% G418^r colonies. In the fourth experiment, 20–30% of CB progenitors were G418^r, indicating significant heterogeneity between individual CB samples with respect to infection efficiency. Com-
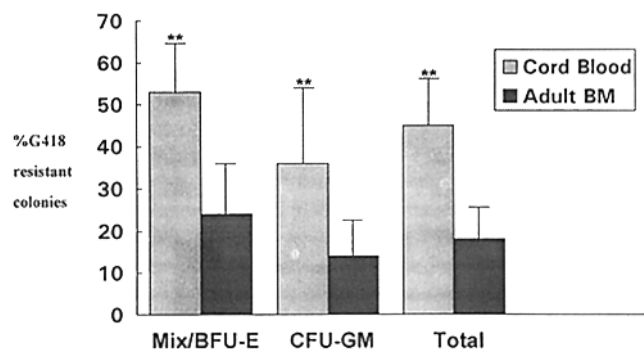
Figure 2. Infection efficiency of CB and ABM-derived committed progenitor cells by cocultivation. Mean ± SD of six independent experiments using TKNEO virus. (* *) Significant differences (p <0.01) between CB and ABM. Background G418-resistant colonies were <1% in all experiments (see text for details).

Table 1. *Infection Efficiency of CB Progenitor Cells Using Various Infection Protocols and TKNEO Vector*

Protocol	Mix/BFU-E	CFU-GM	Total
Coculture	53 ± 12* (6)‡	36 ± 17 (6)	45 ± 12 (6)
Supernatant on fibroblasts	36 ± 18 (3)	18 ± 1 (3)	32 ± 13 (3)
Supernatant on Sl⁴-h220	23 ± 11 (4)	11 ± 7 (4)	19 ± 12 (4)
Supernatant	12 ± 18 (4)	8 ± 10 (4)	11 ± 15 (4)
	3 ± 2 (3)	3 ± 2 (3)	3 ± 2 (3)

* Percent G418 resistant colonies (mean ± SD), background G418^r colonies <1%, see text.
‡ Number of experiments performed.

Table 2. *Infection Efficiency of CB CFU-Mix Cells Using PGK-mADA Vector*

Protocol	Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5
Coculture	10/10*	10/10	–	–	12/12
Supernatant on fibroblasts	–	–	11/12	10/10	12/16
Supernatant on Sl⁴-h220	–	–	16/17	10/10	14/16
Supernatant	–	4/11	–	4/12	3/15

* Number of mADA expressing colonies/total colonies analyzed.

bining results from all four experiments, supernatant infection of CB produced 11 ± 15% G418^r progenitors compared with the generation of 45 ± 12% G418^r colonies by six consecutive CB samples using the cocultivation protocol for infection.

In contrast, supernatant infection protocols that included stromal elements demonstrated improved gene transfer efficiency. As seen in Table 1, supernatant infection on irradiated allogeneic fibroblasts was two to three times more efficient than supernatant infection in the absence of stromal cells, with 32 ± 13% of infected progenitors growing in the presence of G418. Supernatant infection efficiency on the stromal cell line, Sl⁴-h220, was intermediate between supernatant infection without stromal cells and the coculture method and was similar to infection on allogeneic fibroblasts.

Infection of Progenitor Cells with PGK-mADA Vector. The expression of murine ADA from the PGK-mADA virus can be detected by electrophoretic separation of human and murine isozymes and in situ gel analysis. Such analysis is possible on large, single progenitor colonies grown in methylcellulose. Previous work has demonstrated that murine hematopoietic cells infected with PGK-hADA (human) retrovirus express high levels of the transferred ADA cDNA in vivo in transplant recipients (5, 22, 23, 27). Similarly, initial experiments using human ABM cells during this study showed that individual PGK-mADA virus infected progenitor colonies expressed murine ADA at levels up to 10-fold higher than the endogenous human ADA protein (data not shown). Therefore, to stringently analyze transfer efficiency, we considered progenitor colonies transduced only if expression of the transferred mADA was equal to or greater than endogenous human ADA levels (see Fig. 4). As expected, the efficiency of gene transfer into CFU-Mix progenitors using the high titer PGK-mADA virus was substantially better than with the TKNEO vector, irrespective of the infection protocol used (Table 2). Supernatant infection with PGK-mADA virus yielded ~25% mADA expressing committed progenitors (Table 2, expts. 2, 4, and 5) compared to 11% with TKNEO

virus. In contrast, all infection protocols including stromal cells (expts. 3–5) and cocultivations (expts. 1, 2, and 5) yielded nearly 100% mADA expressing progenitor colonies.

Infection of CB and Bone Marrow LTC-IC. Primitive hematopoietic cells capable of initiating sustained growth in in vitro cultures (LTC-IC) have been suggested to be a more primitive progenitor or stem cell phenotype in human bone marrow (29). We have utilized this culture method to compare the efficiency of retroviral transduction between more primitive ABM and CB cell populations. CB LTC-IC–derived colonies were seen at a frequency of 140–480/10^6 input cells. CB LTC-IC–derived colonies were frequently very large (>1,000 cells), in contrast to ABM LTC-IC colonies, which were mostly small CFU-GM–like colonies. Analysis of gene transfer into LTC-ICs using the low titer TKNEO virus with cocultivation (Fig. 3) demonstrated significantly higher numbers of G418^r CB versus ABM-derived colonies from 3-wk-old long-term cultures and from CB versus ABM LTC-IC–derived colonies at 5 wk. Whereas supernatant infection
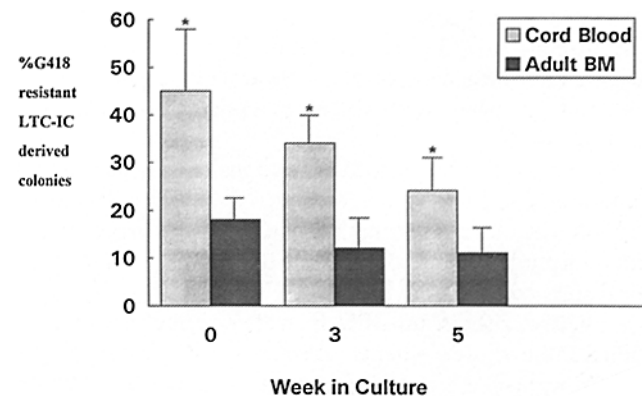
Figure 3. Infection efficiency of CB and ABM primitive cells, including LTC-IC, using TKNEO virus by cocultivation. Mean ± SD of G418^r colonies for committed progenitor cells (week 0, six experiments), for colonies derived from long-term cultures at week 3 (three experiments), and from LTC-IC (week 5, four experiments). (*) Significant difference (p <0.05) between CB and ABM.

Table 3. *Infection Efficiency of CB Primitive Cells, Including LTC-IC, Using Various Infection Protocols and TKNEO Vector*
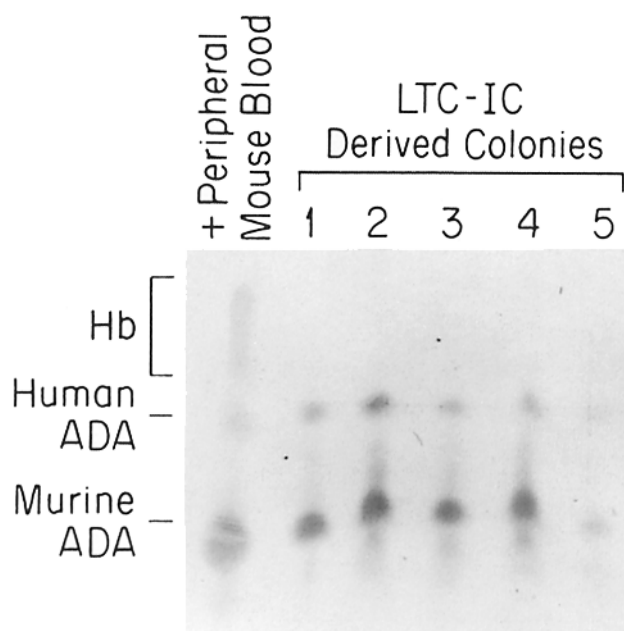
	Time in LTMC		
Protocol	Day 0	Week 3	Week 5
Coculture	45 ± 12* (6)‡	34 ± 6 (3)	24 ± 7 (4)
Supernatant on fibroblasts	32 ± 13 (3)	8 ± 8 (3)	10 ± 7 (3)
Supernatant on Sl⁴-h220	23 ± 11 (4)	9 ± 8 (3)	2 ± 3 (3)
Supernatant	11 ± 15 (4)	4 ± 3 (4)	5 ± 4 (4)

* Percent G418-resistant colonies (mean ± SD), background G418ʳ colonies <1%, see text.
‡ Number of experiments performed.

Figure 4. Expression of murine ADA in CB LTC-IC–derived colonies by in situ enzyme analysis. Location of hemoglobin (*Hb*), human ADA, and murine ADA are noted. (*Left lane*) Peripheral blood from a mouse transplanted with bone marrow infected with PGK-hADA vector (23) to show location of both isozymes.

was generally ineffective in transducing LTC-ICs, supernatant infection in the presence of stromal cells, especially allogeneic fibroblasts, was associated with larger numbers of resistant LTC-IC–derived colonies assayed at 5 wk (Table 3). Analysis of LTC-IC infected with the PGK-mADA virus again clearly demonstrates increased efficiency of infection of these primitive cells using coculture (Table 4). In addition, expression analysis demonstrated high levels of murine ADA protein in the colonies obtained from 5-wk-old LTMC. Fig. 4 shows the level of mADA expression in five representative colonies derived from CB LTC-IC. In each colony, murine and human ADA are easily distinguished and the level of murine ADA expressed from the transferred ADA cDNA exceeds human endogenous ADA. The level of expression of mADA in LTC-IC–derived colonies was similar to that shown in Fig. 4 in a second independent experiment (using two separate CB samples).

Comparison of Cell Cycle Activity in CB and Bone Marrow Committed Progenitors. Integration of the retroviral genome into chromosomal DNA occurs in actively dividing cells (30). Since previous investigators have noted that a large fraction

of myeloid progenitors (CFU-GM) derived from CB are in active cell cycle (20, 21), we directly compared the cell cycle activity of CB progenitors with ABM before and after in vitro exposure to growth factors used in prestimulation (Fig. 5). The data demonstrate that the prestimulation effectively increases the number of all progenitors in cycle. However, we were unable to demonstrate any clear differences in the number of progenitors in cycle from CB compared to ABM either before or after in vitro cultivation.

Discussion

The availability of prenatal diagnosis for a variety of severe genetic diseases, such as SCIDs due to ADA deficiency, and

Table 4. *Infection Efficiency of CB Primitive Cells, Including LTC-IC, Using PGK-mADA Vector*

Expt.	Protocol	Time in LTMC		
		Day 0	Week 3	Week 5
1	Coculture	10/10*	ND	7/11
	Supernatant	4/11	ND	0/6
2	Coculture	12/12	14/14	11/11
	Supernatant	3/15	0/8	1/9

* Number of mADA expressing colonies/total colonies analyzed.
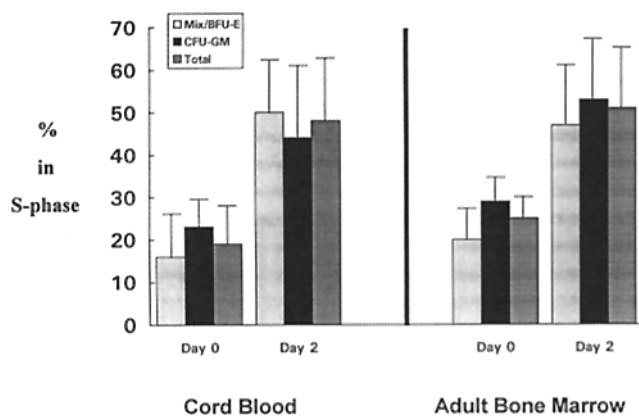
Figure 5. Percentage of cells in S-phase by ^{3}[H]thymidine suicide method. Values represent mean ± SD of six independent experiments determined before (*Day 0*) and after (*Day 2*) prestimulation in growth factors (see text for details).

the increasing success in gene transfer protocols may allow future attempts at early intervention of devastating illnesses by somatic gene therapy. Transplantable CB cells, which are available at the time of delivery from every child, are a potential important resource for hematopoietic stem cells, and the feasibility of large scale collection and storage of these cells is currently being evaluated (Emanuel, D., personal communication). We have examined the potential use of these CB primitive hematopoietic cells as targets for the introduction of new genetic material via recombinant retroviral vectors. Using two vectors differing in titer, design, and expressible sequences, we demonstrate that gene transfer efficiency is significantly higher into CB committed progenitors and primitive LTC-IC compared with ABM cells.

Several factors are known to increase the efficiency of gene transfer into primitive hematopoietic cells via retroviral vectors (31). As previously reported, using ABM cells (32, 33), this study found that higher recombinant viral titer was associated with higher gene transfer efficiency into CB cells, albeit in our study, the comparison is made with different vectors. As first demonstrated by our laboratory (27) and Bodine et al. (34) using adult murine bone marrow cells, prestimulation of CB cells with cytokines before infection was associated with high transduction efficiency. In this report, we demonstrate that high frequency of gene transfer after preincubation of CB cells was associated with a significant increase in the number of committed progenitors that are in active cell cycle after this in vitro exposure to growth factors. The increase in cell cycle status may be important in gene transduction, since retroviral-mediated genome integration is believed to be dependent on cell cycle status (30). However, unlike previous reports of CB CFU-GM colonies, we found no higher frequency of cycling committed progenitor cells in CB versus ABM. Our data are in agreement with a more

recent analysis of CB cycling rates (35). We have also demonstrated that infection efficiency of CB cells is optimized by using cocultivation, but significant improvement over supernatant infection can be seen when allogeneic stroma is included in the infection protocol. These data are similar to data reported previously by other investigators (12, 36) using ABM.

We are currently unable to explain the differences in gene transfer efficiency into CB versus ABM, although this difference may be related to other differences in the biology of CB versus ABM hematopoietic cells. CB cells can be amplified in vitro in response to cytokines to a greater extent than ABM progenitor cells (18, 37). In addition, CB-derived multilineage progenitor cells (CFU-Mix) have been shown to generate secondary colonies upon replating at significantly higher frequencies than ABM CFU-Mix (38). Similarly, a distinct CB CD34^{3+} (bright)-derived colony type has been shown to be capable of multiple replatings in vitro (39). No similar cell has been described from ABM marrow. Hows et al. (19) have demonstrated that CB contains larger numbers of LTC-IC with higher proliferative capacity than comparable numbers of adult nucleated bone marrow cells. These data suggest that primitive CB cells may have higher proliferative and self-renewal potential than primitive cells in ABM. However, as with ABM, it remains unclear what effect ex vivo expansion of CB progenitor cells will have on the ultimate ability of such cells to achieve long-term reconstitution.

Previously, our laboratory (5, 22) has demonstrated long-term and stable expression of introduced human ADA in mice receiving transplants of bone marrow stem cells transduced with the PGK-hADA retroviral vector. The present study demonstrates high-level mADA expression in human committed progenitors and in colonies derived from the more primitive LTC-IC cell after infection with a similar virus. Although the relationship of the LTC-IC and the reconstituting stem cell has not been established, these data are consistent with studies in primates receiving CD34$^+$ bone marrow cells transduced with this same retroviral vector in which high levels of murine ADA expression have been demonstrated in vivo after hematopoietic reconstitution (40). It is interesting to note that this work in primates had failed to transduce reconstituting stem cells by coculture methods, whereas infection on genetically modified stromal cells led to long-term expression in transplanted monkeys (Bodine, D., and D. A. Williams, manuscript in preparation). Taken together, these data suggest that the PGK-ADA vector, which contains only one expressible genetic sequence and lacks a dominant selectable marker, may be useful for transduction of human bone marrow in gene therapy protocols. CB progenitor and stem cells may provide a unique target cell population for transduction using this and other retroviral vectors in attempts at curative somatic gene therapy.

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