Cloning and Functional Analysis of the Rhesus Macaque ABCG2 Gene

FORCED EXPRESSION CONFRS AN SP PHENOTYPE AMONG HEMATOPOIETIC STEM CELL PROGENY IN VIVO

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Hematopoietic cells can be highly enriched for repopulating ability based upon the efflux of the fluorescent Hoechst 33342 dye by sorting for SP (side population) cells, a phenotype attributed to expression of ABCG2, a member of the ABC transporter superfamily. Intriguingly, murine studies suggest that forced ABCG2 expression prevents hematopoietic differentiation. We cloned the full-length rhesus ABCG2 and introduced it into a retroviral vector. ABCG2-transduced human peripheral blood progenitor cells (PBPCs) acquired the SP phenotype but showed significantly reduced growth compared with control. Two rhesus macaques received autologous PBPCs split for transduction with the ABCG2 or control vectors. Marking levels were similar between fractions with no discrepancy between bone marrow and peripheral blood marking. Analysis for the SP phenotype among bone marrow and mature blood populations confirmed ABCG2 expression at levels predicted by vector copy number long term, demonstrating no block to differentiation in the large animal. In vitro studies showed selective protection against mitoxantrone among ABCG2-transduced rhesus PBPCs. Our results confirm the existence of rhesus ABCG2, establish its importance in conferring the SP phenotype, suggest no detrimental effect of its overexpression upon differentiation in vivo, and imply a potential role for its overexpression as an in vivo selection strategy for gene therapy applications.

Hematopoietic stem cells (HSCs) are widely studied because of their self-renewal capacity leading to potential utility in therapeutic applications such as bone marrow transplantation and gene therapy. However, successful HSC-based gene therapy is encumbered by the relatively low frequency of genetically modified cells with repopulating ability attainable using current methods. A better understanding of the mechanisms that control HSC self-renewal and differentiation could allow improvement in gene transfer techniques, and the ability to identify and purify these cells unambiguously is essential to identifying genes that are involved specifically in regulating HSC activity. Goodell et al. (1) described a primitive cell phenotype within the murine marrow characterized by the efflux of the DNA dye, Hoechst 33342, which is highly enriched for repopulating ability. This fraction, termed the side population for its characteristic appearance when viewed by flow cytometric methods, has subsequently been detected in the marrow of both nonhuman and human primates and represents 0.05% of adult BM cells in humans and 0.03% of adult BM cells in nonhuman primates (2). SP cells possess remarkable repopulating ability, with single “Tip” SP cells engrafting 96% of lethally irradiated mice (3). Furthermore, SP cells have been identified in a number of nonhematopoietic organs (4–7), suggesting that the phenotype may be useful in identifying stem cells of other organ types.

Recently, a half-transporter known as the breast cancer resistance protein (Bcrp1), also known as ABCG2 (8, 9), was identified in human breast cancer cells and characterized as a novel stem cell transporter (4, 10–12), and the molecular basis for the SP phenotype has been attributed to expression of ABCG2, not the multidrug resistance-associated protein (MRP1) gene (13) or MDR1 (4). Molecular studies revealed that the ABCG2 transporter is expressed in a highly regulated manner in the hematopoietic compartment, with expression in the primitive cell fraction and sharp down-regulation following lineage commitment (10). These studies collectively support a role for ABCG2/Bcrp-1 in the maintenance of hematopoietic stem cells and suggest that its forced overexpression may prove useful as a strategy for improving the contribution of genetically modified cells toward hematopoiesis as an in vivo selection strategy.

Zhou et al. (4) reported that overexpression human ABCG2 in mouse bone marrow cells significantly blocked hematopoietic development leading to speculation that ABCG2/Bcrp-1 expression may play a role in early stem cell self-renewal by blocking differentiation. In contrast, overexpression of the related membrane transporter, human MDR1, resulted in HSC expansion and a myeloproliferative disease in mice (14, 15). Furthermore, deletion of the murine homologue of ABCG2/Bcrp1 resulted in a marked decrease in the number of SP cells in the marrow, yet stem cell activity was not affected (16). Still, little is known...
regarding ABCG2 function in the hematopoietic compartment in primates.

We therefore sought to determine the effects of forced expression of the ABCG2 gene in the nonhuman primate model, a model with proven relevance to human hematopoiesis. Toward this end, we cloned the full-length rhesus ABCG2 (Rh-ABCG2) cDNA and constructed an RD114-pseudotyped MFGS-ABCG2 retroviral vector along with a control vector. The present study demonstrates the first direct evidence that overexpression of ABCG2 in large animal bone marrow stem cells does not interfere with hematopoietic stem cell maturation in vivo. Furthermore, forced expression confers protection to mitoxantrone in vitro. These results suggest that overexpression of ABCG2 may be feasible as an in vivo selection strategy for gene therapy applications.

**EXPERIMENTAL PROCEDURES**

**Rhesus ABCG2 Cloning**—Rhesus ABCG2 (Rh-ABCG2) cDNA was amplified by reverse transcription-PCR from rhesus kidney, which expresses high levels of ABCG2. Total RNA was isolated from rhesus

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**Fig. 1.** Rh-ABCG2 vector. The full-length Rh-ABCG2 cDNA was cloned into the NcoI and BamHI sites of MFGS with no other payload. RD114-pseudotyped vector was prepared from a stable producer derived from the FLYRD packaging cell line.

**Fig. 2.**

A, expression of Rh-ABCG2 among transduced human CD34+ cells. Closed histograms represent gp91<sup>1H<sub>iso</sub></sup> (control) transduced cells, and open histograms represent Rh-ABCG2-transduced cells. B, expression of human gp91<sup>1H<sub>iso</sub></sup> among transduced human CD34+ cells. Closed histograms represent Rh-ABCG2-transduced (control) cells, and open histograms represent gp91<sup>1H<sub>iso</sub></sup>-transduced cells. C–E, SP cell phenotype analysis among human CD34+ cells transduced with the gp91<sup>1H<sub>iso</sub></sup> vector (0.05%, C), the Rh-ABCG2 vector (56.3%, D), and after inhibition of Hoechst 3342 efflux by FTC for Rh-ABCG2-transduced cells (E). The total cell number after 4 and 8 days of culture (n = 5) (F). The overall percentage of cells expressing CD34 or both CD34 and CXCR4 after 8 days of culture (G) and the absolute number of CD34 or both CD34- and CXCR4-positive cells after 8 days of culture (n = 5) (H).
kidney using RNA STAT-60 (Tel-Test, Friendswood, TX) with DNase and reverse-transcribed to cDNA with the SuperScript™ first strand synthesis system (Invitrogen). Rh-ABCG2 cDNA was amplified with Advantage 2 polymerase (Clontech, Palo Alto, CA) using a series of overlapping primers spanning the entire sequence designed using the published human sequence. Each PCR product was then cloned using the TOPO TA® cloning kit (Invitrogen). All of the sequences were confirmed using an ABI PRISM™310 Genetic Analyzer (Applied Biosystems, Foster City, CA). Each PCR product was then cloned using the TOPO TA® cloning kit (Invitrogen). All of the sequences were confirmed using an ABI PRISM™310 Genetic Analyzer (Applied Biosystems, Foster City, CA). After each clone was cut by the enzyme (SpeI, BshNI, and Eco72I), clones were ligated using the Rapid DNA ligation kit (Roche Applied Science) to make full-length Rh-ABCG2 cDNA. Sequence homology was 97% at the cDNA level and 96% at the protein level compared with that of human ABCG2 sequence (GenBank™ accession number AY841878).

Generation of an RD114-pseudotyped MFGS-ABCG2 Retrovirus Producer Cell Line—To generate MFGS-Rh-ABCG2 vectors, Rh-ABCG2 cDNA was amplified with primers containing the 3’ BamHI site (5’-TTCGGGATCCCAAAGTGCTTCTTTTT-3’) and the 5’ PciI site (5’-GAGACATGTCTTCCAGTAATGT-3’) with Platinum Pfx DNA polymerase (Invitrogen). The PCR product was then cloned using the TOPO TA® cloning kit (Invitrogen) and inserted into the NcoI-BamHI cloning site of MFGS as depicted in Fig. 1 (17, 18). VSVG-pseudotyped MFGS-ABCG2 producer cells were made by transfection of 3T3 cells with the MFGS-ABCG2 plasmid, gag, pol, and env (VSVG) genes by calcium phosphate coprecipitation. RD114-pseudotyped MFGS-ABCG2 was then prepared by transducing the FLYRD18 packaging cell line (19) with supernatant from VSVG-pseudotyped MFGS-Rh-ABCG2 (20). Transduced cells were sorted after immunofluorescent staining with a fluorescein isothiocyanate (FITC)-conjugated anti-human ABCG2 monoclonal antibody (clone 5D3; Stem Cell Technologies, Vancouver, Canada) on a FACSVantage flow cytometer (Becton Dickinson, San Jose, CA). The brightest cells were plated at limiting dilution. A high titer producer clone was then selected, and a stable virus-transduced cell line was established. The control vector was constructed in an
identical fashion and expresses the human gp91phox, a phagocyte oxidase subunit deficient in X-linked chronic granulomatous disease (20).

**Table I**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Apheresis products</th>
<th>Column yield</th>
<th>Total cell dose infused</th>
<th>Cell dose infused from each transduced fraction</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ABCG2</td>
</tr>
<tr>
<td>3545</td>
<td>7.50</td>
<td>6.5</td>
<td>15.7</td>
<td>69.6</td>
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<td>3549</td>
<td>7.85</td>
<td>2.7</td>
<td>95.5</td>
<td>95.7</td>
</tr>
</tbody>
</table>

*a* × 10^9 cells.

*b* Number of CD34+ cells obtained after selection × 10^9.

*c* CFU transduction efficiency (%).

*d* Total number of cells infused after transduction × 10^7.

*e* Total number of cells from each fraction infused after transduction × 10^7.

Rh-ABCG2 Confers SP Phenotype

**TABLE I**

**Transplant characteristics**

**Analysis of Transgene Expression and Hoechst Efflux Studies by Flow Cytometry**—1 × 10^6 transduced cells were resuspended in phosphate-buffered saline with 0.5% bovine serum albumin. After blocking of surface Fc receptors, the cells were incubated with FITC anti-human ABCG2. Human gp91phox expression was determined by indirect staining with murine monoclonal antibody 7D5 followed by FITC-conjugated goat antimouse IgG antibody (Jackson Immunoresearch Laboratories, West Grove, PA) (20). Transduced cells were analyzed by flow cytometry using a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson) to determine the percentage of transgene expression in human CD34+ PBPCs. All of the antibody incubations were carried out for 30 min on ice. Rhesus and human SP cell analyses were performed exactly as described (2). At least 100,000 events were collected for each analysis. For the inhibitor experiments, fumitremorgin C (FTC) (the kind gift of S. Bates, National Institutes of Health), which is an ABCG2-selective inhibitor (23, 24), was added to the cells at a final concentration of 10 μM. The cells were then incubated at 37 °C for 20 min before Hoechst staining. For antibody staining of Hoechst-stained cells, all of the cells were resuspended into 100 μl of Hank's balanced saline solution, 1 mM HEPES, 2% fetal calf serum (HBSS + ) medium and incubated with FITC-conjugated rhesus lineage panel antibodies comprising CD3 (Becton Dickinson), CD20 (Becton Dickinson), and CD11b (Milenyi Biotec). After washing, the cells were resuspended into 0.5 ml of HBSS + containing 2 μg/ml propidium iodide and analyzed on a FACScan vantage flow cytometry (Becton Dickinson).

Rhesus Cell Harvesting, Transduction, and Transplantation—Young rhesus macaques were housed and handled in accordance with the guidelines set by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, and the protocol was approved by the Animal Care and Use Committee of the NHLBI, National Institutes of Health. Mobilized PB cells were harvested by apheresis and CD34+-enriched as previously described (25). For each animal, CD34-selected cells were divided into two equal fractions and transduced with either the ABCG2 or gp91phox vector as described above with concentrated vector supernatant (RQ3545, 3.5-fold; RQ3549, 5-fold) and infused fresh. The animals received lethal myeloablative and supportive care as previously described (26).

**Δ to CFU Assay**—An aliquot of cells pre- and post-CD34 selection as well as an aliquot of cells post-transduction were plated in MethoCult H4230 (Stem Cell Technologies) as previously described (27). Twenty-four colonies from each of the post-transduction samples plated were analyzed for the presence of the transferred gene by PCR (27).

**Quantitative Real Time PCR Analysis**—PB and BM samples were obtained at regular time points following recovery. Granulocyte (G) and mononuclear cells (MNC) were isolated as previously described (27). The contribution to engraftment by ABCG2 and gp91phox vector-transduced cells was determined by real time PCR analysis with a SYBER® Green reporter as previously described (21, 28) using vector-specific primers. The primer sequences for the ABCG2 vector were: forward primer (5'-GAACTCCCTTTGATCCCAT) and reverse primer (5'-TCCGGTCAATTTGAGTG), using the standard setting of 40 cycles with an annealing temperature of 60 °C by real time PCR using SYBER® Green PCR core reagents (PE Biosystems Warrington, UK). For the gp91phox vector, internal primers were used by amplifying the purified products of the outer reaction. The primer sequences for the gp91phox vector for the outer reaction were: forward primer (5'-CAGCAGACTAAGAC) and reverse primer (5'-CGGCTAGGACCAGATGGAAG), and the cycle conditions were 95 °C for 1 min, 60 °C for 0.5 min, and 72 °C for 2 min for eight cycles amplified with Advantage 2 polymerase PCR kit. The primer sequences for the inner reaction were: forward primer (5'-GAATGGGTCCGACCGTGCAGGC) and reverse primer (5'-CCAAACCATGAACTTTGAGTG), using the standard setting of 40 cycles with an annealing temperature of 80 °C.

**Cytotoxicity Assay**—Rhesus CD34+ PBPCs were seeded at a density of 2 × 10^6 cells/well in 24-well plates and transduced under identical conditions with either the ABCG2 or control vector (n = 2). Mitoxantrone was then added at 0, 10, 25, 50, or 100 nM, and the cells were cultured for an additional 4 days. The percentage of living cells remaining was determined by flow cytometry after the addition of propidium iodide (2 μg/ml). To determine whether selection for ABCG2 expressing cells occurred during exposure to mitoxantrone, transduced cells were analyzed for ABCG2 expression after culture in the presence of 25 μM mitoxantrone and compared with those cultured in its absence.

**Statistical Analysis**—The data are presented as the means ± standard deviation. The statistical significance of the data was determined by Student’s t test. The significance level was set at 0.05.

**RESULTS**

Concentrated RDI14-MFGS-ABCG2 and gp91phox Achieves High Transduction of Human CD34+ PBPC, and Rh-ABCG2-transduced CD34+ Cells Adopt an SP Phenotype—Gene transfer rates to CFU of greater than 70% were estimated by colony PCR analysis (data not shown). Fig. 2 (A and B) shows representative FACS analyses demonstrating high level expression of both Rh-ABCG2 and gp91phox. Fig. 2 (C–E) demonstrates analysis for the SP phenotype among mobilized human PB CD34+ cells transduced with either the control or ABCG2 vector or that performed with FTC. Among gp91phox-transduced PBPCs—
duced cells, SP cells were detectable at only 0.03%. In contrast, greater than 50% of cells transduced with Rh-ABCG2 were of the SP phenotype, and these results matched that estimated by flow using the anti-ABCG2 antibody. Functional expression of Rh-ABCG2 was confirmed by blocking with FTC. These data demonstrate that forced expression of the Rh-ABCG2 gene results in a marked increase in the number of SP cells.

**Gene Transfer of the Rh-ABCG2 Gene to Human Mobilized Peripheral Blood CD34+ Cells Results in Diminished Growth in Vitro but Does Not Inhibit Colony Formation**—Human CD34+/H11001 PBPCs transduced with the ABCG2 retroviral vector displayed significantly reduced growth when compared with the control vector (absolute numbers at day 4 were 7.75 ± 1.99 versus 10.24 ± 2.57 × 10⁵, p = 0.050; those at day 8 were 7.67 ± 2.54 versus 17.83 ± 6.64 × 10⁵, p = 0.0024 for ABCG2- and gp91phox-transduced cells, respectively; n = 5; Fig. 2F). The overall percentage of cells expressing CD34 or both CD34 and CXCR4 was, however, higher after transduction with the Rh-ABCG2 vector compared with the control (CD34, 44.5 ± 11.7% versus 28.8 ± 12.58%; CD34 and CXCR4, 22.0 ± 8.60% versus 11.2 ± 7.14%; n = 5; Fig. 2G). On the other hand, the reduction in cell growth over the Rh-ABCG2 transduction resulted in equivalence the absolute number of CD34/CXCR4+ cells between the Rh-ABCG2 and control transduced fractions (CD34+, 3.7 ± 1.7 versus 4.3 ± 2.7 × 10⁵; CD34/CXCR4+, 1.8 ± 0.97 versus 1.6 ± 1.1 × 10⁵; n = 5; Fig. 2H). Additionally, there were no differences noted between the Rh-ABCG2- and gp91phox-transduced cells with respect to plating efficiency (52 ± 4.8 versus 43.8 ± 6.1 total CFU/500 cells plated for ABCG2- and gp91phox-transduced cells, respectively; n = 3; p = 0.076). These findings demonstrate that Rh-ABCG2 overexpression results in diminished growth in vitro but does not inhibit the differentiation of hematopoietic colony-forming progenitors.

**RD114-MFGS-Rh-ABCG2- and gp91phox-transduced Rhesus PB CD34 Cells Contribute Equally to Hematopoietic Reconstitution after Lethal Irradiation**—The transplantation schema is depicted in Fig. 3A. Table I summarizes the apheresis yield, CD34 yield, CD34 enrichment, CFU transduction efficiency, and infused cell number. Approximately 95% of the separated cells were CD34+ (data not shown). Both animals engrafted rapidly, with neutrophil counts of more than 500 cells/μl by day 10. Fig. 3(B and C) shows amplification results and a standard curve for the dilutions of a known Rh-ABCG2 copy number into a background of nontransduced rhesus peripheral blood by real time PCR. Fig. 4 shows the real time PCR estimates for the overall level of engraftment by genetically modified cells in both animals. The percentage of cells containing either vector reached stable levels of 0.05–0.4% in both animals with no difference between engraftment levels by either the Rh-ABCG2 or control vector transduced cells 7 months after transplantation.

We examined the engraftment levels by Rh-ABCG2- and gp91phox-transduced cells within the BM CD34+ cell fraction 5 months after transplantation and compared with other populations to determine whether Rh-ABCG2-transduced cells were blocked in their hematopoietic development. As shown in Table II, we observed no difference in marking by Rh-ABCG2-trans-
duced cells within the BM CD34+ population as compared with BM CD34− cells and PB MNCs or Gs in both animals.

Peripheral Blood Cell Progeny of Rh-ABCG2-transduced Cells Adopt an SP Phenotype—We then examined bone marrow and peripheral blood for cells of the SP phenotype after engraftment. SP cells were easily detectable in the BM (2.75%) and PB (0.65%) cells in animal 2 (Fig. 5) at 4 weeks post-transplantation. To determine the role of vector-encoded Rh-ABCG2 in the observed SP phenotype, BM and PB SP and non-SP fractions were sorted to estimate the percentage of Rh-ABCG2 and gp91phox vector-containing cells by real time PCR. PCR estimates for Rh-ABCG2 were 85.2 and 100% for bone marrow and peripheral blood SP cells, respectively. On the other hand, PCR estimates for Rh-ABCG2 were 3.8 and 2.4% for the non-SP BM and peripheral blood samples, respectively, confirming the observed SP phenotype derived from vector-encoded Rh-ABCG2 expression.

Finally, we investigated the differentiation and maturation potential for Rh-ABCG2-transduced cells in both animals. Fig. 6 shows the analysis of PB MNCs and Gs in the second animal 7 months after transplantation. The frequency of SP cells in PB...
and consisted of 96.1% CD11b+ and 10.4% CD20+ B cells, a distribution similar to that of whole PB MNCs. The frequency of SP cells in PB granulocytes was 0.12% and consisted of 96.1% CD11b+ myeloid cells. CD3, CD20, and CD11b positive SP and non-SP cells were subsequently sorted and evaluated for the percentage containing the Rh-ABC2G vector by real time PCR (Fig. 6C and Table III). CD3 and CD20 positive SP cells were estimated at 100% vector containing. CD11b cells were estimated at 17.3% vector containing. Only 0.04% of non-SP CD11b+ cells were vector containing. The lower fraction of CD11b+ cells estimated to contain vector encoding Rh-ABC2G suggests that the CD11b region contains cells that express endogenous Bcrp/ABCG2 such as natural killer cells (4, 10–12). Although marking was lower in the first animal, the frequency of SP cells among the mature cell populations was similar to that observed in the second (Table III). These results demonstrate that Rh-ABC2G-transduced cells can differentiate into mature T, B, and myeloid cells in vivo while expressing the transgene with no disturbance in the frequency of these cell types in the circulation.

**In Vitro Protection from Mitoxantrone by Rh-ABC2G Expression**—Rhesus PBPCs transduced with the Rh-ABC2G vector demonstrated relative protection from mitoxantrone (Fig. 7A). Additionally, Rh-ABC2G expressing cells were selectively protected, with 77% of cells expressing ABCG2 after exposure to mitoxantrone at 25 nM versus 32% of those not exposed (Fig. 7B).

**DISCUSSION**

The ATP-binding cassette (ABC) transporters are transmembrane transporters that mediate the transfer of a broad spectrum of substrates across the cell membrane. P-glycoprotein, or MDR1, was the first such transporter identified and mediates resistance to a number of antineoplastic agents used in the treatment of cancer (29). This property stimulated interest in the incorporation of MDR1 into retroviral vectors to allow in vivo selection (30–34). Previous studies have demonstrated that retroviral vectors expressing MDR1 conferred the SP phenotype to transduced and expanded murine bone marrow cells (14); yet a myeloproliferative syndrome was noted in some recipient mice. Additionally, MDR1a/1b knockout mice contain normal numbers of bone marrow SP cells, arguing that expression of MDR1a/1b is not required for the SP phenotype (11). Based upon inhibition studies, Zhou et al. (4) discovered that the related ABC transporter, Bcrp1/ABC2G, is a molecular determinant of the SP phenotype. ABC2G is preferentially expressed in primitive hematopoietic progenitors (10), and this property has suggested a role in the isolation of stem cells (12). Curiously, forced ABC2G expression in murine bone marrow significantly blocked differentiation, suggesting that ABCG2/Bcrp expression may play a role in early stem cell self-renewal (4). On the other hand, Bcrp−/− mice demonstrated that ABCG2 functionality is not required for normal stem cell function (16). To determine the effects of forced ABCG2 expression within the hematopoietic stem cell compartment of large animals, we cloned the rhesus ABCG2 gene to allow investigation in the large animal model. The use of Rh-ABC2G in the rhesus macaque autologous transplantation model avoids potential confounding variables such as immune recognition of cells expressing xenogeneic genes.

We obtained high transduction rates to CFU from human and rhesus CD34+ PBPCs with both the Rh-ABC2G and control vectors, and more than 50% of ABCG2-transduced cells demonstrated an SP phenotype. Similar to that observed in the murine model (4), an apparent block to differentiation was noted as the total cell number achieved during culture was significantly reduced in the Rh-ABC2G-transduced fraction when compared with the control, and yet CD34/CXCR4+ cell numbers were preserved. This apparent block has implications on the potential for exploiting forced ABCG2 expression as an in vivo selection strategy. We thus examined the frequency of Rh-ABC2G- and gp91phox-transduced cell progeny among BM and peripheral blood cell populations in the autologous transplantation model. Should ABCG2 expression exerts its effect on early stem cell self-renewal by blocking differentiation, a contribution by Rh-ABC2G-transduced cells should be restricted to the more primitive compartment within the bone marrow, with a discrepancy in the contribution toward mature peripheral blood progeny when compared with that of the control gp91phox-transduced fraction. However, no such discrepancy was observed. We detected cells of the SP phenotype among multiple lineages in both animals 7 months after transplantation, confirming that Rh-ABC2G-transduced rhesus stem cells differentiate into mature cells from CD34+ cells. Furthermore, PCR analysis of sorted SP cells demonstrated that the peripheral blood SP phenotype was derived from expression of the transferred Rh-ABC2G vector. Moreover, we observed no difference in engraftment levels between Rh-ABC2G- and gp91phox-transduced cells, indicating that ABCG2 expression has no adverse effect on hematopoietic differentiation in large animals. These results represent the first direct evidence that ABCG2 expression does not block differentiation in large animals. Why these results differ from that observed in the murine model may relate to differences in stem cell biology between the mouse and the nonhuman primate. Although evidence suggests that hematopoietic stem cell number is in fact preserved in mammals, the greater hematopoietic requirement places a higher proliferation and differentiation demand on HSCs of larger animals (35). It therefore follows that the regulation of large animal (and human) hematopoiesis is more complex, and redundancies may exist in higher animals. Certainly, differences between the small and large animal have been observed with respect to the first of the ABC transporters to be tested in both models. The myeloproliferative syndrome observed in mice after transplantation of hematopoietic stem cells transduced with MDR1 (15) was not observed in our rhesus macaque model (36, 37). Differences in gene transfer efficiency into mouse versus rhesus HSCs might explain this result, because the oligocolonal expanded stem cell population found in mice showed high proviral copy number, and expression at high

<table>
<thead>
<tr>
<th>Animal Cell fraction</th>
<th>SP cells a (%)</th>
<th>Lineage b (%)</th>
<th>ABCG2 marking c (%)</th>
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<tbody>
<tr>
<td>BM MNC</td>
<td>0.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PB MNC</td>
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<tr>
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a Percentage of SP cells within each fraction from BM or PB.
b Percentage of SP or NonSP cells positive for indicated lineage marker (CD3, T cells; CD20, B cells from MNCs; CD11b, myeloid cells from Ga). c Estimate of percentage marking within each fraction by real time PCR.
levels may have been required to produce the phenotype. Further, copy number is generally restricted to one/cell using standard retroviral vectors in the rhesus macaque model (36, 38). However, ABCG2 expression was confirmed by the presence of cells of the SP phenotype in the circulation of the rhesus macaque (39). Although the role of ABCG2 in hematopoiesis remains to be fully elucidated, our current understanding would support a role in protecting stem cells from damage resulting from exposure to naturally occurring toxic substrates, including those that have been developed for therapeutic application in cancer chemotherapy (40–42), and this natural role could be exploited for in vivo drug selection using ABCG2 substrates for clinical gene therapy applications.

REFERENCES

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