In Vivo Selection of Antifolate-Resistant Transgenic Hematopoietic Stem Cells in a Murine Bone Marrow Transplant Model

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ABSTRACT

Currently, low levels of stable gene transfer into hematopoietic tissues of large animals and humans continues to limit the clinical application of gene therapy. One strategy for overcoming this problem is to selectively expand, in vivo, the population of successfully gene-modified cells. Recent work has shown that nucleoside transport inhibition in combination with antifolates can be used to select in vivo for hematopoietic stem cells expressing drug-resistant dihydrofolate reductase (DHFR). In this study we investigated whether trimetrexate (TMTX) and the nucleoside transport inhibitor prodrug nitrobenzylmercaptopurine ribose phosphate (NBMPR-P) can be used to select for tyr22-variant DHFR expressing transgenic hematopoietic cells in a murine bone marrow transplant model. Our results indicate that 40 mg/kg TMTX and 20 mg/kg NBMPR-P can be used in combination to expand transgene-positive progenitor cells 3- to 4-fold immediately following drug administration. In addition, long-term progenitor populations were expanded 2- to 3-fold in primary recipients, to approximately 5 months following drug administration. Secondary transplants conducted with marrow from primary recipients 5 months following drug administration revealed a statistically significant selective expansion of transgene-positive cells in the spleens and peripheral blood of these animals. No such expansion was observed in groups of mice treated with TMTX alone or NBMPR-P alone. We conclude that TMTX + NBMPR-P can be used to selectively expand transgenic tyr22-variant DHFR expressing murine hematopoietic stem cells in vivo.

Gene transfer into hematopoietic tissue has the potential for treatment of a variety of diseases in humans. For many of these disorders, stable gene transfer into hematopoietic stem cells would provide the most benefit to patients. Unfortunately, gene transfer into hematopoietic tissues with stably integrating vectors in large animal models and humans has been reported to be very inefficient, limiting its potential application (Chu et al., 1998). One possible approach to overcoming this low level of stable gene transfer is to selectively expand, in vivo, those cells into which gene transfer has been successfully achieved.

Several systems have been described that allow for in vivo selection of hematopoietic cells in mice. The human multidrug resistance gene (MDR-1) (Sorrentino et al., 1992) confers resistance to several chemotherapeutic agents such as anthracyclines, Vinca alkaloids, podophyllotoxins, paclitaxel (Taxol), and actinomycin D. DNA alkyltransferase genes confer resistance to 1,3-bis-(2-chloroethyl)-1-nitrosourea (Davis et al., 1997). A third system utilizes drug-resistant forms of the enzyme dihydrofolate reductase (DHFR) in combination with the antifolates methotrexate (MTX) and trimetrexate (TMTX) (Allay et al., 1997, 1998). These studies have provided proof of principle for in vivo selection, and have provided a foundation upon which to explore this strategy.

DHFR catalyzes the reduction of folate and dihydrofolate to tetrahydrofolate in mammals (Blakley and Benkovic, 1984). Reduced folates serve as cofactors for enzymes involved in thymidylate synthesis and de novo purine synthesis (Blakley and Benkovic, 1984). Rapidly dividing cells are therefore reliant upon reduced folates for cell division, and depletion of reduced folates by drugs such as the antifolates MTX and TMTX results in cell death. Substitutions in DHFR, particularly at position 22 or 31, render the enzyme resistant to antifolates while retaining a variable level of catalytic activity (Morris and McIvor, 1994). This has led to the use of variant DHFRs as dominant selectable markers in vitro (Simonsen and Levinson, 1983) and for chemoprotection
of animals and selection in vivo (Williams et al., 1987; Corev et al., 1990; May et al., 1995; James et al., 1997; Allay et al., 1998). However, the potency of antifolates can be interrupted by the salvage of preformed nucleosides, thus providing nucleotide precursors that enter metabolic pathways distal to the site of action of the antifolates (Cabral et al., 1984; Nelson and Drake, 1984). This circumvention of antifolate toxicity can be reversed, however, by the use of agents such as dipyriddamide or nitrobenzylmercaptopurine riboside (Cabral et al., 1984; Nelson and Drake, 1984; Allay et al., 1997; Warlick et al., 2000) to inhibit nucleoside transport. Allay et al. (1997) found that nucleoside transport inhibition could be used to sensitize murine hematopoietic stem cells to the antifolate TMTX in vivo. This led directly to the demonstration that murine hematopoietic stem cells transduced with the tyr22 variant of human DHFR could be selected in vivo using the combination of TMTX and NBMPR-P (Allay et al., 1998).

In this study we corroborated the results of Allay et al. (1998) by demonstrating in vivo selection of murine hematopoietic stem cells in mice transplanted with bone marrow transgenic for drug-resistant DHFR. Primary recipient mice treated with TMTX + NBMPR-P demonstrated a 2- to 3-fold increase in transgene signal in bone marrow, spleen, and peripheral blood when assayed by quantitative Southern analysis 5 months following drug administration. Analysis of secondary transplant recipients derived from primary recipient mice treated with TMTX + NBMPR-P demonstrated increased levels of transgene-positive cells in marrow, peripheral blood, and spleens of these mice, indicative of expanded transgenic stem cells in the primary recipients, despite using a dose of TMTX less than one-third the dose of previously reported studies (Allay et al., 1998).

**Materials and Methods**

**Mice.** Female transgenic FVB/N mice expressing the tyr22 variant of murine DHFR were used in this study as a source of drug-resistant marrow for bone marrow transplant studies and have been previously described (James et al., 1997). Age-matched normal FVB/N male mice were used as a source of nontransgenic competitor marrow in bone marrow transplant studies. Recipient mice in bone marrow transplant studies were normal FVB/N female mice aged 8 to 10 weeks. Normal FVB/N mice were obtained from the National Institutes of Health facility at Frederick, MD. All mice were provided with food and water ad libitum. All animal work was approved by the University of Minnesota Animal Care Committee and carried out in accordance with University of Minnesota animal care guidelines.

**Bone Marrow Transplantation.** For primary bone marrow transplants, donor marrow was harvested from tyr22 DHFR transgenic mice (line 11 (James et al., 1997)) and normal mice by flushing tibiae and femurs with Iscove’s modified Dulbecco’s medium without supplements. Marrow from transgenic and normal donors was then expanded transgenic stem cells in the primary recipients, despite using a dose of TMTX less than one-third the dose of previously reported studies (Allay et al., 1998).

**Drug Administration.** NBMPR-P and TMTX in the form of trimetrexate glucuronate were kindly provided by Drs. Brian Sorrentino and James Allay (St. Jude’s Medical Center, Memphis, TN). Both drugs were solubilized in sterile water. TMTX was administered at doses ranging from 20 to 100 mg/kg as a subcutaneous injection, and NBMPR-P was administered at 20 mg/kg as an i.p. injection. Phosphate-buffered saline (PBS) was given as an i.p. injection.

**In Vitro Hematopoietic Cell Culture.** Total marrow cells were plated in 0.9% base methylcellulose (H4100) (Stem Cell Technologies, Vancouver, BC, Canada) supplemented with 30% newborn calf serum (Summit Biotechnology, Ft. Collins, CO), 0.5% bovine serum albumin (Stem Cell Technologies), 0.1 mM 2-mercaptoethanol (Invitrogen, Carlsbad, CA), 5% Hemostim (Stem Cell Technologies), 3 U/ml recombinant human erythropoietin (R & D Systems, Minneapolis, MN), and in the presence or absence of 50 nM MTX (±) amethopterin; Sigma, St. Louis, MO). Between 2 and 5 × 10⁶ cells were plated per 3-cm plate. Samples for each mouse were plated in triplicate at each drug concentration, incubated at 37°C and 5% CO₂ in a humidified chamber, and counted on day 14. Results are reported as the percentage of transgene engraftment, calculated by determining the ratio of the number of colonies in the presence versus absence of MTX for each sample and dividing it by the ratio of colonies in the presence versus absence of MTX for a line 11 transgenic control mouse (defined as 100%) and multiplying by 100. Standard deviations for each triplicate plating were less than 20% unless otherwise noted.

**White Blood Cell Counts.** Peripheral blood was obtained from drug-treated primary recipient mice upon sacrifice 24 h following the last drug administration. Blood was collected and diluted, and red cells were lysed using the Unopette collection system (BD Biosciences, Franklin Lakes, NJ) as per the manufacturer’s instructions. Total white blood cells were then counted using a hemocytometer.

**Southern Analysis and Quantitation.** DNA was extracted from marrow and spleen as previously described (James et al., 1997). DNA was isolated from peripheral blood samples using the Puregene DNA Isolation Kit (Pharmacia, Piscataway, NJ) as per the manufacturer’s instructions. DNA samples were digested with BglII restriction enzyme (Invitrogen), separated on 0.8% agarose, and transferred to a positively charged Nytran Plus (Schleicher & Schuell, Keene, NH) membrane. The blots were then probed with a [³²P]dCTP (Amerham Biosciences, Arlington Heights, IL)-labeled fragment generated by polymerase chain reaction from the murine DHFR gene, which hybridizes to both the endogenous and transgenic DHFR sequences, and which has been described previously (James et al., 1997). Quantitation of radioactive signals from Southern blots was carried out using a 445Si PhosphorImager (Molecular Dynamics, Sunnyvale, CA). The percentage of transgene engraftment was calculated as the ratio of transgenic DHFR signal to endogenous DHFR signal in each sample divided by the ratio of transgenic DHFR signal to endogenous DHFR signal in a transgenic control animal (defined as 100%) × 100.

**Statistical Analysis.** Statistical comparison between different groups of animals was carried out using analysis of variance (Statview v.5.0.1; SAS Institute Inc., Cary, NC). Probability values less than 0.05 were considered significant.

**Results**

The purpose of this study was to investigate whether TMTX administration with or without nucleoside transport inhibition could be used to mediate the selective outgrowth of marrow cells transgenic for tyr22 drug-resistant DHFR. We first tested the toxic effects of TMTX in female FVB/N mice. Animals were given subcutaneous injections of TMTX at doses ranging from 20 to 100 mg/kg once daily for 5 days, monitoring the animals for survival and weight loss. We found that in this strain of mice, wild-type animals suc-
cumbed to TMTX at doses of 80 mg/kg and greater in approximately 8 days (Table 1). Of the TMTX doses tested, 60 mg/kg was the maximally tolerated dose resulting in survival of most animals following a 5-day treatment course (Table 1). To test whether the nucleoside transport inhibitor prodrug NBMPR-P exacerbated TMTX toxicity in FVB/N mice, TMTX was administered at 40 and 60 mg/kg subcutaneously for 5 days with or without 20 mg/kg NBMPR-P administered as an i.p. injection, again monitoring daily for survival and weight loss. As shown in Table 2, NBMPR-P exacerbated the toxicity of TMTX in FVB/N mice. Whereas one-third of the mice given 60 mg/kg TMTX alone survived, all of the mice administered TMTX at this dose died when NBMPR-P was coadministered (Table 2). NBMPR-P administration also reduced the survival of animals administered 40 mg/kg TMTX from three of three to two of three (Table 2). Based upon these observations, TMTX and NBMPR-P were administered at 40 and 20 mg/kg, respectively, in the experiments described below.

To generate a cohort of mice for in vivo selection studies, bone marrow transplants were conducted with marrow containing 10% tyr22 DHFR transgenic cells. Female FVB/N mice were transplanted with 1x10^7 total bone marrow cells, 1x10^6 of which were derived from a syngeneic transgenic mouse line that expresses the tyr22-variant murine DHFR. The remaining competitor cells were derived from normal FVB/N mice. Primary recipient mice were allowed to engraft for 4 months (Fig. 1). Following engraftment, recipient mice were divided into four groups and administered either PBS, TMTX alone at 40 mg/kg, NBMPR-P alone at 20 mg/kg, or TMTX and NBMPR-P for 5 days. Both TMTX-administered groups exhibited hematopoietic toxicity, as indicated by a significant reduction in white blood cell count observed between animals administered PBS (9.3 x 10^3 cells/µl) and either TMTX alone (2.9 x 10^3 cells/µl (**p < 0.01)) or TMTX + NBMPR-P (2.3 x 10^3 cells/µl (**p < 0.01)). We thus concluded that the doses of TMTX used, either alone or in combination with NBMPR-P, demonstrated appreciable hematopoietic toxicity in FVB/N mice and therefore may render early progenitors or stem cells susceptible to selection.

To determine the effect that the various drug treatments had on the level of transgenic DHFR-expressing cells present in primary recipient mice, three animals from each group were sacrificed 24 hours following drug administration. Peripheral blood, spleen, and bone marrow were harvested from the animals, isolating DNA for quantitative Southern analysis. Protection from antifolate treatment was apparent from the higher percentage of DHFR transgene-positive cells in the marrow of animals treated with either TMTX (**p < 0.01 versus NBMPR-P alone or PBS) or TMTX + NBMPR-P (p < 0.05 versus NBMPR-P alone or PBS) (Table 3). TMTX- and TMTX + NBMPR-P-treated mice exhibited an average of 20 to 30% transgene-positive cells in the marrow, a 3- to 4-fold increase over the level of transgene engraftment detected in animals administered PBS or NBMPR-P alone (Table 3). No relative increase in the level of transgene was observed in the spleen or peripheral blood (data not shown). Thus, although TMTX given with or without NBMPR-P was toxic to the hematopoietic system as a whole, the protective effects of the tyr22 DHFR transgene, at least in the period immediately following drug administration, were more pronounced in the marrow than in the spleen or peripheral blood.

The effect of drug administration on more primitive progenitor cells in primary recipients was determined in animals allowed to recover for 5 months following drug administration. DNA was isolated from marrow, spleen, and peripheral blood, and then subjected to quantitative Southern analysis as described under Materials and Methods. In the marrow, there was a statistically significant increase in

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### Table 1

<table>
<thead>
<tr>
<th>TMTX Dose</th>
<th>Median Survival</th>
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<tr>
<td>PBS</td>
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<tr>
<td>20</td>
<td>&gt;15</td>
</tr>
<tr>
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<td>8</td>
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*a n = 3; b n = 6.

### Table 2

<table>
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<th>Drug Dose</th>
<th>Fraction of Mice Surviving for 14 Days</th>
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<tbody>
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<td>40 TMTX</td>
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</tr>
<tr>
<td>40 TMTX + NTI</td>
<td>2/3</td>
</tr>
<tr>
<td>60 TMTX</td>
<td>1/3</td>
</tr>
<tr>
<td>60 TMTX + NTI</td>
<td>0/3</td>
</tr>
</tbody>
</table>

*NBMPR-P (20 mg/kg) was administered in addition to TMTX.

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### Table 3

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Group Averages*</th>
<th>S.D.</th>
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<tr>
<td>TMTX</td>
<td>29.8</td>
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<tr>
<td>NBMPR-P</td>
<td>9.1</td>
<td>±1.2</td>
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<tr>
<td>PBS</td>
<td>7.4</td>
<td>±5.5</td>
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<tr>
<td>TMTX + NBMPR-P</td>
<td>22.3</td>
<td>±4.8</td>
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</tbody>
</table>

*Percentage of transgene engraftment levels were determined as described under Materials and Methods, and means and standard deviations for each group of three mice are shown.

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*Fig. 1.* Experimental scheme. Schematic representation of experiments. BMT, bone marrow transplant.
the level of transgene-positive cells in TMTX + NBMPR-P-treated mice relative to all other groups (Fig. 2). The average level of transgene-positive cells for animals treated with both drugs was 10.4% compared with averages of 4.9% for PBS-treated mice, 4.8% for NBMPR-P-treated mice, and 4.1% for mice treated with TMTX alone (Table 4) (*p < 0.05 versus all three groups), suggesting that TMTX + NBMPR-P selectively expanded tyr22 transgenic progenitor cells.

Marrow obtained from three animals in each group of drug-administered primary recipients was also plated into methylcellulose culture to assay for colony formation in the absence and presence of 50 nM MTX. There was a trend toward higher engraftment levels in the TMTX + NBMPR-P-treated mice compared with the other groups, although these differences were not statistically significant (Table 4). In general, the frequency of drug-resistant colony formation for any given animal correlated well with the level of transgene engraftment observed in the marrow and peripheral blood as determined by quantitative Southern analysis (Fig. 3). Furthermore, since a selective effect was maintained for 5 months after drug administration, this implies that observed increases in transgene level occurred at the level of primitive progenitors and, perhaps, stem cells.

Mice treated with TMTX + NBMPR-P contained higher levels of DHFR transgene than did PBS-treated mice in the spleen (*p < 0.05), and there was a trend toward higher engraftment levels in peripheral blood, although this difference was not statistically significant (Fig. 3). A significant difference in transgene level was also observed in the spleen between mice treated with TMTX + NBMPR-P and mice treated with TMTX alone (**p < 0.01); however, no significant difference was observed in the spleen between animals treated with NBMPR-P alone and those treated with TMTX + NBMPR-P. Assessed engraftment levels were consistent from tissue to tissue for any given animal. Mice that demonstrated high levels of engraftment in the marrow generally also exhibited high levels in the two peripheral compartments (peripheral blood and spleen) that were assayed, although the absolute level of engraftment was somewhat lower in the spleen than in either the marrow or peripheral blood (Fig. 3; Table 4). The observed increases in transgene-positive cells in marrow, spleen, and peripheral blood of mice treated with TMTX + NBMPR-P relative to other groups suggested preferential contribution from very early transgenic progenitor or stem cells to long-term hematopoiesis in these animals.

To further evaluate in vivo antifolate-mediated selection at the stem cell level, secondary transplants were conducted using marrow obtained from animals 1 day or 5 months following administration of PBS, TMTX, NBMPR-P, or both TMTX + NBMPR-P. Recipients of marrow obtained 1 day after drug administration were allowed to engraft for 7 months before harvesting bone marrow, spleen, and peripheral blood. Low levels of transgenic cell engraftment (group averages < 3%) were observed by quantitative Southern analysis of peripheral blood, marrow, and spleen in all groups of these secondary recipients (data not shown), and drug-resistant colony formation of marrow cells plated in methylcellulose culture was negligible (data not shown). Furthermore, there was no detectable difference in transgene engraftment level or drug-resistant colony formation among any of the treatment groups. Transgenic stem cell selection was thus not observed in secondary recipients when transplanted immediately following drug administration.

In contrast, secondary recipients of marrow obtained from animals 5 months after drug administration exhibited substantially higher levels of transgenic cell engraftment. In marrow, the average transgene engraftment levels for all of the groups ranged from 10 to 13% except for the PBS-administered control group, which was 4.7% (Table 4). Interestingly, however, average transgene engraftment levels in the spleen and peripheral blood for the TMTX + NBMPR-P group were 31.2 and 21.1%, respectively (Table 4). By comparison, the NBMPR-P, PBS, and TMTX-only groups each exhibited lower mean engraftment levels (Table 4). Statistically significant differences were observed between the TMTX + NBMPR-P group and the PBS group (*p < 0.05 in the spleen, *p < 0.05 in peripheral blood) and between the TMTX + NBMPR-P group and the NBMPR-P-only group (*p < 0.05 in spleen, *p < 0.05 in peripheral blood). Assuming a baseline engraftment level of 5% (the approximate transgene engraftment level for PBS-treated primary recipients at 5 months), four of five peripheral blood samples and five of five spleen samples exhibited at least a 2-fold increase in transgene-positive cells in the TMTX + NBMPR-P group (Table 4). Marrow cells from these secondary transplant recipients were also plated in methylcellulose culture in the presence and absence of MTX to assay for the presence of drug-resistant progenitor cells. Although statistical significance was not observed between any of the groups, two of five secondary recipients from the TMTX + NBMPR-P group demonstrated transgene engraftment levels approximately 2-fold higher than that achieved by any other mouse in any group, reaching 50% in one case (Table 4). This suggested selective expansion of drug-resistant progenitors in these animals. The relative levels of transgene-positive cells determined by colony-forming assay again correlated well with quantitative Southern analyses for individual mice. Taken together, the engraftment levels assessed by Southern analysis and the results from analysis of drug-resistant colony formation in samples obtained from secondary transplant recipients indicated effective in vivo selection of variant DHFR transgenic hematopoietic stem cells using a combination of TMTX + NBMPR-P.

![Fig. 2. Southern hybridization analysis of bone marrow from primary recipient mice 5 months after drug administration. Blots were probed with a fragment of the murine DHFR gene that identifies both the endogenous DHFR gene (EN) and the DHFR transgene (TG) as described under Materials and Methods. Drug treatments are indicated above the lanes. TMTX was administered at 40 mg/kg and NBMPR-P was administered at 20 mg/kg. All drugs were administered daily for 5 days. wt, wild-type FVB/N control mouse; tyr22, line 11 tyr22 DHFR transgenic mouse control.](image-url)
Table 4 is presented in the document, titled "Transgene engraftment levels in primary and secondary bone marrow transplant recipients." The table is formatted in a two-column layout with sub-columns. Each row contains data points for different conditions or groups, with columns for Southern, Colony Formation, Marrow, Spleen, and Peripheral Blood. Percentages and numerical values are tabulated for each category.

### Discussion

In this study, we investigated the use of TMTX with and without nucleoside transport inhibition to selectively expand hematopoietic stem cells expressing a drug-resistant tyr22-variant DHFR transgene. Increased levels of transgene-positive cells were documented in marrow, spleen, and peripheral blood in primary recipients. This enrichment, approximately 2- to 3-fold over controls, was stable for up to 5 months after drug treatment and translated into an increase in the number of drug-resistant progenitors as assayed by colony formation in methylcellulose culture. Secondary recipients transplanted with marrow harvested 5 months after treatment of donor mice with TMTX + NBMPR-P exhibited increased levels of transgene-positive cells in the marrow, spleen, and peripheral blood in comparison to control animals. We therefore conclude that the combination of TMTX + NBMPR-P selectively expands tyr22 DHFR-positive hematopoietic stem cells.

One of the current problems in gene therapy is the challenge of expressing genes of interest in clinically significant numbers of cells. Adenovirus vectors are able to transduce large numbers of cells, but only transiently. In addition, the immunogenicity of these vectors makes readministration problematic. Retroviral vectors such as those derived from Moloney murine leukemia virus integrate into host cells, but efficient integration requires host cell replication. Because hematopoietic stem cells replicate infrequently, stable transduction with Oncornavirus vectors is problematic. This has led to the investigation of stem cell transduction with alternative vectors such as adenov-associated virus (Ponnazhagan et al., 1997)- and human immunodeficiency virus-based vectors (Miyoshi et al., 1999). Modification of stem cell culture conditions may also ultimately improve transduction frequency (Kiem et al., 1998).

Another potential strategy for increasing the frequency of genetically modified cells is a selective strategy that enriches for those cells expressing a selectable function introduced by genetic modification. There are several advantages to in vivo versus in vitro selection strategies. Hematopoietic progenitors can be expanded and selected in culture with the use of cytokines, but this in vitro approach can result in the loss of primitive stem cell phenotype (Hutchings et al., 1998). In contrast, in vivo selective expansion of genetically modified cells minimizes the length of time in culture and thus the potential loss of primitive progenitors. Theoretically, genetically modified cells could be selectively expanded to therapeutic levels from even a very low level of engraftment. Recent data from this laboratory and others have shown that severe my-
In this paper we corroborate the results of Allay et al. (1997, 1998) in C57BL/6J mice. Determining the minimal effective dose of antifolate will be important to minimize toxicity of selective treatment. We have also observed that FVB/N mice transgenic for variant DHFR or transplanted with variant DHFR transgenic marrow can tolerate significantly higher doses of antifolate than normal animals (May et al., 1995; James et al., 1997).

The marrow of primary recipients receiving TMTX or TMTX + NBMPR-P demonstrated a 3- to 4-fold higher transgene signal compared with PBS- or NBMPR-P- administered mice when assayed 1 day after drug administration. The resulting average levels of transgene-positive cells for antifolate-administered mice were between 20 and 30%. However, when marrow was examined in primary recipient mice 5 months after drug administration, transgene engraftment levels for TMTX + NBMPR-P-treated mice had decreased to an average of 10.5%, and had decreased to 4.1% in mice treated with TMTX alone. The level of transgene-positive cells observed immediately after antifolate treatment most likely represents selection of committed hematopoietic cells. Over time, however, the committed progenitors presumably exhausted their replicative potential and were lost from the population, resulting in a lower transgene engraftment level indicative of the level of transgene-positive early-progenitor or stem cells present.

The tyr22-transgenic DHFR mice used as a source of drug-resistant marrow for these studies have been described previously (line 11) (James et al., 1997). Despite the presence of approximately 24 copies of the transgene per cell, the amount of messenger RNA derived from the transgene in bone marrow was found to be about 20% that of the endogenous DHFR message level as determined by ribonuclease protection assay (James et al., 1997). Spencer et al. (1996) found that the in vivo level of myeloprotection from TMTX was correlated with the tyr22 DHFR proviral copy number in peripheral blood cells of mice transplanted with transduced marrow. It is likely that higher expression levels of the variant DHFR, achievable either through transduction of marrow using viral vectors with strong heterologous promoters or a different line of transgenic mice expressing a higher transgene level, might result in more effective chemoprotection and a greater degree of in vivo selection. Thus far, only the tyr22 form of DHFR has been tested for selection in vivo. Other forms of DHFR which confer greater resistance to antifolates may prove even more effective for in vivo selection than tyr22.

In this study we have shown that transgenic hematopoietic stem cells expressing tyr22 DHFR can be selectively expanded in vivo using the antifolate TMTX and the nucleoside transport inhibitor prodrug NBMPR-P. Further studies are being conducted to address optimal drug dosing schedules as well as the minimum level of transgene engraftment that can be successfully expanded in vivo in this system. Application of this strategy could be beneficial for treatment of genetic...
diseases by gene transfer into hematopoietic cells. In vivo expansion of such a population of gene-modified cells expressing both a selective and a therapeutic gene could result in an improved therapeutic benefit. Furthermore, increased levels of gene-modified cells achieved through in vivo selection may provide a greater degree of chemoprotection, allowing for more aggressive antitumor chemotherapy. In vivo selection is thus a promising strategy in the continuing maturation of human gene therapy.

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References


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