ones, expression of the functional γc alone may not account for the low-level, but substantial engraftment of this lineage. In this study, we used the Ly5.1/5.2 congenic system to distinguish the donor cells from the recipient cells. Recently van Os *et al* reported that the Ly5-congenic BM engrafted poorer than glucose phosphate isomerase-congenic cells following minimal conditioning.[14] Consistent with their report, our immunocompetent Ly5.1 animals rejected as many as 1 x 10^7 Ly5.2 BM cells following non-myeloablative BMT, presumably by T and NK cell-mediated mechanisms. Lacking T and NK cells, Ly5.1 X-SCID mice allowed low-level engraftment of Ly5.2 myeloid cells, and outgrowth of Ly5.2 lymphocytes boosted by γc-mediated growth advantage. Thus, one should consider the immunogenicity of Ly5 antigens when assessing engraftment following different conditioning regimens and the immunological status of the host.

The results of this report provide strong support for the hypothesis that the selective growth advantage of lymphoid precursors with a functional γc played a critical role in the gene therapy trial for X-SCID. The growth advantage seems to be direct and much stronger than the benefit from the adenosine deaminase (ADA) gene transfer. The functional ADA gene can confer a survival advantage on lymphoid precursors, so that the transduced lymphocytes accumulated in the ADA-deficient SCID patients.[15] However, the advantage seems to be inadequate to expand gene-corrected cells for clearing metabolic toxicity, so that only minimal immune reconstitution was achieved in the stem cell-targeted ADA gene transfer trials. Hence, there is a need to maximize, or boost, the growth advantage of the transduced cells for successful gene therapy of diseases other than X-SCID and perhaps Jak3-deficient SCID.

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Titles and legends to Figures

Figure 1 Lymphocyte reconstitution in the X-SCID recipient mice following BMT. Ly5.1 X-SCID recipients were transplanted with either 10^5 (circles), 10^6 (triangles) or 10^7 (diamonds) Ly5.2 wild-type BM cells, and the peripheral blood was drawn monthly to enumerate total lymphocytes (panel **a**), $CD4^+$ T cells (panel **b**), $CD8^+$ T cells (panel **c**), surface $IgM^+/B220^+$ B cells (panel **d**), and $NK1.1^+/TCR\beta^-$ NK cells (panel **e**).

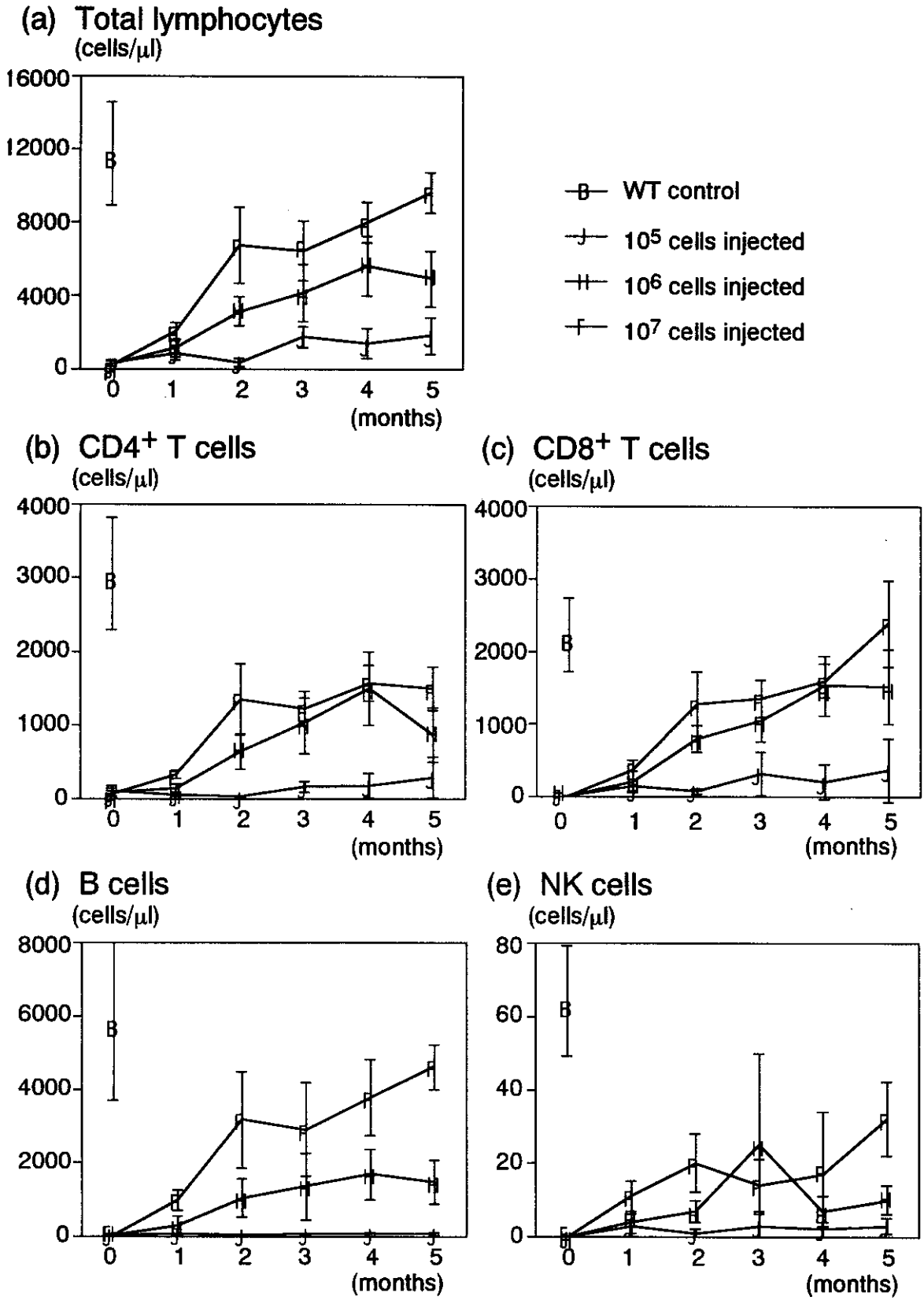
Figure 2 Leukocyte chimerism in the wild-type (WT) and X-SCID recipients following BMT. Unconditioned Ly5.1 WT and Ly5.1 X-SCID mice were transplanted with Ly5.2 WT BM cells (10^5, 10^6 or 10^7 cells). Five months following BMT, the donor cell chimerism was calculated by flow cytometry. Shaded bars, hatched bars, and dark bars represent the recipients transplanted with 10^5 cells, 10^6 cells and 10^7 cells, respectively.

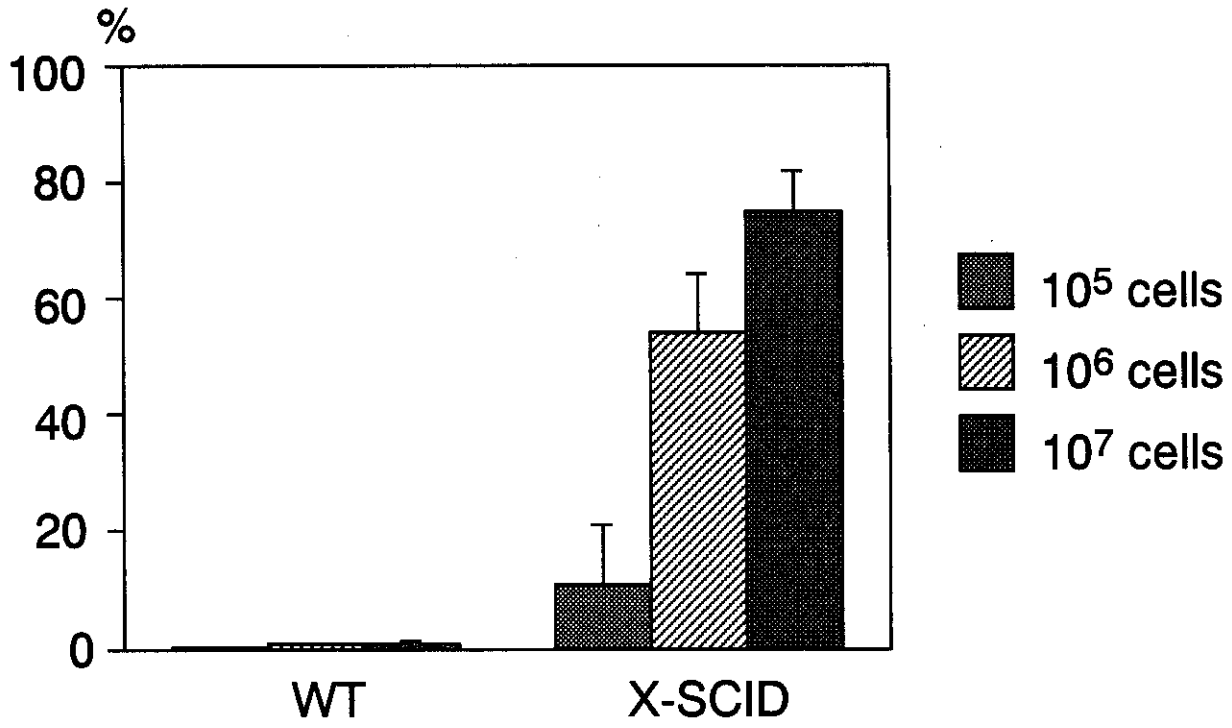
Figure 3 Donor cell contribution to each hematopoietic lineage in the X-SCID recipients. Five months after BMT, peripheral blood cells were stained with lineage-specific antibodies and a Ly5.2 antibody. Donor cell chimerism was calculated by flow cytometry for granulocytes (Gr, $Gr1^{high}/Mac1^{+}$), monocytes (Mo, $Gr1^{low\text{-}mid}/Mac1^{+}$), CD4 T cells (CD4, $CD4^{+}/CD8^{-}$), CD8 T cells (CD8, $CD4^{-}/CD8^{+}$), B cells (B, $IgM^{+}/B220^{+}$) and NK cells (NK, $NK1.1^{+}/TCR\beta^{-}$). Shaded bars, hatched bars, and dark bars represent the recipients transplanted with 10^5 cells, 10^6 cells and 10^7 cells, respectively.

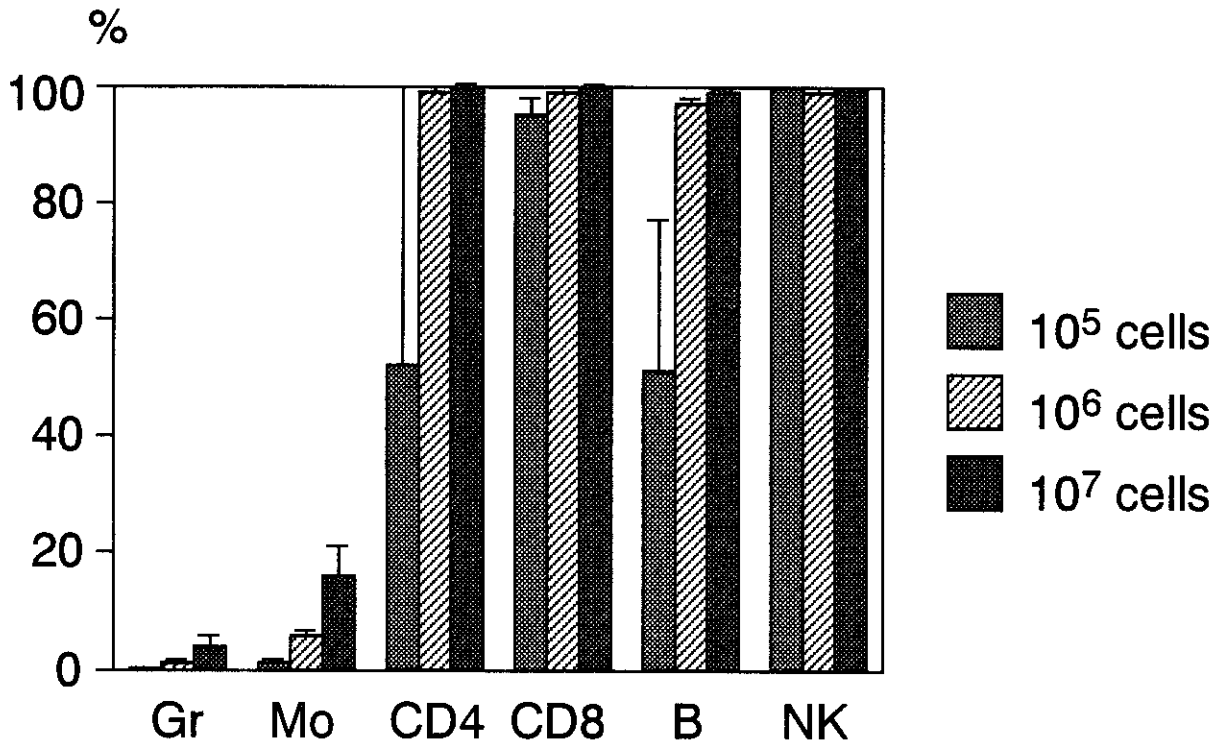
Table 1 Lymphocyte reconstitution in X-SCID mice after BMT

Mouse and treatment	Post-BMT (months)	Lymphocyte count (mean ± s.d., cells/μl) Total	$CD4^+$ T	$CD8^+$ T	B	NK
WT control (n = 12)	0	13,933 ± 3,423	3,053 ± 760	2,221 ± 512	5,838 ± 2,135	64 ± 15
X-SCID + 10^5 WT cells (n = 4)	0	234 ± 50	99 ± 81	4 ± 1	14 ± 13	0 ± 0
	5	1,814 ± 999	281 ± 278	359 ± 433	65 ± 40	3 ± 1
X-SCID + 10^6 WT cells (n = 5)	0	290 ± 105	84 ± 58	4 ± 3	19 ± 19	0 ± 0
	5	4,909 ± 1,504	859 ± 375	1,512 ± 513	1,478 ± 593	10 ± 4
X-SCID + 10^7 WT cells (n = 6)	0	229 ± 61	68 ± 36	4 ± 1	9 ± 2	0 ± 0
	5	10,209 ± 1,122	1,599 ± 290	2,469 ± 592	5,076 ± 617	34 ± 10

WT, wild-type; X-SCID, X-linked SCID


(a) Total lymphocytes
(cells/μl)
16000
12000
8000
4000
0
0 1 2 3 4 5
(months)
WT control
10^5 cells injected
10^6 cells injected
10^7 cells injected
(b) CD4+ T cells
(cells/μl)
4000
3000
2000
1000
0
0 1 2 3 4 5
(months)
(c) CD8+ T cells
(cells/μl)
4000
3000
2000
1000
0
0 1 2 3 4 5
(months)
(d) B cells
(cells/μl)
8000
6000
4000
2000
0
0 1 2 3 4 5
(months)
(e) NK cells
(cells/μl)
80
60
40
20
0
0 1 2 3 4 5
(months)


%
100
80
60
40
20
0
WT
X-SCID
10^5 cells
10^6 cells
10^7 cells


%
100
80
60
40
20
0
Gr
Mo
CD4
CD8
B
NK
10^5 cells
10^6 cells
10^7 cells

In vivo expansion of transduced murine hematopoietic cells with a selective amplifier gene

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Abstract

Although hematopoietic stem cells are ideal targets for genetic therapy, gene transfer into this compartment has been inefficient. To circumvent this obstacle, we have developed 'selective amplifier genes (SAGs)' to expand transduced hematopoietic cells. By encoding a fusion protein between the granulocyte colony-stimulating factor receptor and the estrogen binding domain, a prototype SAG conferred an estrogen-responsive growth on murine hematopoietic progenitors. We have refined the SAG system to achieve 4-hydroxytamoxifen (Tm)-specific cell growth with minimum differentiation, and herein evaluated its efficacy *in vivo*. Murine bone marrow cells were transduced with a retrovirus vector coexpressing a modified SAG and the enhaned green fluorescent protein (EGFP), and transplanted into myeloablated mice. After hematopoietic reconstitution, Tm challenge to the transplasnts resulted in a significant increase of $EGFP^+$ leukocytes (21 ± 4% to 27 ± 5%), while the unchallenged animals had decreased $EGFP^+$ frequencies (21 ± 5% to 10 ± 3%). After keeping the difference for 3 months, a secondary transplantation was carried out. Again Tm challenge resulted in an increse of $EGFP^+$ cells (16 ± 4% to 35 ± 3%), contrasting to a decrease in the untreated animals (22 ± 4% to 12 ± 4%), and the difference was significant for more than 3 months. A detailed lineage study showed a preferential expansion of $EGFP^+$ cells in granulocytes and monocytes following Tm injection. These results showed that the long-term repopulating cells were transduced with the SAG and expandable *in vivo*, and that the myeloid progeny was particularly responsive.

Introduction

Hematopoietic stem cells (HSCs), with an indefinite self-renewal capacity and pluripotency to differentiate into all the hematopoietic lineages, are attractive targets for gene therapy. Candidate disorders include monogenic diseases such as hereditary immunodeficiencies, hemoglobinopathies and lysosomal storage diseases, as well as infectious diseases and cancer. In contrast to the efficient gene transfer into murine HSCs and successes in treating mouse disease models by oncoretroviral vectors, gene transfer into human HSCs with the same kind of vectors has been extremely inefficient.[1] The main reason for this inefficiency is inability of oncoretoviruses to integrate into nondividing cells such as most human HSCs.[2] Another reason is very poor expression of the receptors for amphotropic retroviruses on the human HSCs.[3] To overcome these obstacles, much interest has been focused on developing vectors capable of integrating into quiescent cells. In particular, lentiviral vectors pseudotyped with vesicular stomatitis virus G-glycoprotein (VSVG) are most promising, with their ability to integrate into nondividing cells and ubiquitous receptor distribution for this envelope.[4]

Besides vector development, an alternative strategy is to select or expand transduced stem cells after gene manipulation and reinfusion into the patient. To this goal, transfer of a drug-resistance gene such as *mdr-1* has been extensively studied,[5] but it may raise a controversy against giving cytotoxic compounds to patients without malignancy. In this regard, we took a less toxic approach by designing a novel system for conferring a direct proliferative advantage on genetically modified cells. This system is based on the ability of a steroid receptor hormone binding domain (HBD) to reversibly activate a fusion counterpart in a steroid-dependent manner.[6] As a prototype fusion, the granulocyte colony-stimulating factor (G-CSF) receptor (GCR) was

fused to the HBD of estrogen receptor (ER), and this fusion protein (GCRER) induced an estrogen-dependent growth in transduced murine progenitors.[7] We termed the hybrid genes for the chimeric molecules between growth factor receptors and HBDs as 'selective amplifier genes (SAGs)', and have further refined this system. Herein we investigated whether SAG-transduced murine hematopoietic cells could be expanded *in vivo*, by administrating a drug specific for the corresponding fusion receptor.

Materials and methods

Plasmids, cells and animals

Construction of SAGs encoding estrogen-responsive chimeric receptors (GCRER and its G-CSF binding site-deleted form, DGCRER) was described, and DGCR was released from pMX/DGCRER as a *Bam*H I-*Pme* I fragment.[7,8] 4-hydroxytamoxifen (Tm)-specific HBD (TmR) was obtained by a polymerase chain reaction on pBS^{+}ER^{TM} (a gift from Drs. G. I. Evan and T. D. Littlewood), adding a 5'-*Pme* I and a 3'-*Sal* I site.[9,10] A retrovirus carrying the encephalomyocarditis virus internal ribosome entry site (IRES) and the enhanced green fluorescent protein (EGFP) gene was also described (MSCV2.2/IRES-EGFP).[11-13] DGCR (*Bam*H I-*Pme*) and TmR (*Pme* I-*Sal* I) was inserted into *Bgl* II/*Xho* I-digested MSCV2.2/IRES-EGFP by trimolecular ligation, resuting in a bicistronic retrovirus MSCV/DGCRTmR-IRES-EGFP. Finally, a Y703F mutation was introduced into MSCV/DGCRTmR-IRES-EGFP using a Transformer Site-Directed Mutagenesis Kit (Clontech, Palo Alto, CA) as described.[14] The resultant vector was designated as MSCV/DY703FGCRTmR-IRES-EGFP (Figure 1).

BOSC23 ecotropic packaging cells (American Type Culture Collection CRL-11554,

Manassas, VA) were maintained in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum (Life Technologies, Grand Island, NY).[15] Retrovirus supernatant was prepared by transient transfection of BOSC23 cells with MSCV/DY703FGCRTmR-IRES-EGFP. Transfection was carried out using LipofectAmine reagent (Life Technologies) following the manufacturer's protocol, and the viral supernatant was harvested 2 days postlipofection. Titration of the vector supernatant was estimated by a simplified RNA dot-blot and hybridization assay along with a reference vector MSCV/EGFP-neo.[13,16]

C57BL/6-Ly5.2 mice were purchased from Clea Japan (Tokyo, Japan). The congenic C57BL/6-Ly5.1 mice were bred in the animal facility of Jichi Medical School (Tochigi, Japan). The mice were maintained and treated following the institutional ethical codes for animal rights.

Bone marrow (BM) transduction and hematopoietic reconstitution

Retroviral transduction of BM cells was carried out following a fibronectin-assisted protocol.[17] C57BL/6-Ly5.2 mice (6 weeks old) were injected intraperitoneally (ip) with 150 mg/kg 5-fluorouracil (Kyowa Hakko, Tokyo, Japan), and BM cells were obtained by flushing femora 2 days postinjection. Low density mononuclear cells were collected using Lympholyte-M (Cedarlane Laboratories, Hornby, Ontario, Canada) and prestimulated for 2 days with 100 ng/mL recombinant rat stem cell factor (provided by Amgen, Thousand Oaks, CA) and 20 ng/mL recombinant human interleukin (IL)-6 (provided by Ajinomoto, Kawasaki, Japan).[18] The prestimulated cells were incubated in the fresh viral supernatant on plates precoated with recombinant human fibronectin fragment CH-296 (RetroNectin; provided by Takara Shuzo, Otsu, Japan) for 2 additional days under the same growth factor condition as for prestimulation.[17] Supernatant infection was repeated two to four times during transduction. The manipulated

cells were recovered using Cell Dissociation Buffer (Life Technologies) and injected into the hosts via the tail vein.

For primary reconstitution, C57BL/6-Ly5.1 mice (8 weeks old) were lethally irradiated (11 Gy; ^{137}Cs using a Gamma Cell 40, Nordion International, Kanata, Ontario, Canada) and intravenously given 2 x 10^5 transduced Ly5.2 BM cells per animal. For secondary reconstitution, fresh BM cells from the primary recipients were pooled and 2 x 10^6 cells were infused into lethally irradiated Ly5.1 recipients.

4-Hydroxytamoxifen (Tm) formulation and administration

Tm (Sigma, St. Louis, MO) was solubilized in ethanol to produce a 25 mg/mL solution, and this solution was diluted with saline containing polyethylene glycol 400 and Tween 80. The final solution for injection contained 5 mg/mL Tm, 20% ethanol, 10% polyethylene glycol 400 and 1.4% Tween 80. For 5 mg/day regimen, 330 ml of the solution was ip injected 3 times daily.

Hematological examinations and flow cytometry

Hematopoietic reconstitution and the impact of Tm administration was assessed by complete blood count and fluorescence-activated cell sorting (FACS). Mouse peripheral blood (100-150 ml) was obtained by tail clipping. A complete blood count was measured with a PC-608 particle counter (Erma, Tokyo, Japan), and the blood smear was Wright-Giemsa stained for leukocyte differential count. Red blood cells in the remainder of the blood samples were lysed with ACK buffer (150 mM NH_4Cl, 12 mM $KHCO_3$, 0.125 mM EDTA) for flow cytometry. To detect EGFP fluorescence, Ly5 congenic antigens and lineage markers, the cells were stained with antibodies specific for Ly5.1, Ly5.2, Gr1, Mac1, CD4, CD8, B220, IgM, T cell receptor b

chain (TCRb) and NK1.1 (all from Pharmingen, San Diego, CA), and analyzed with a FACScan (Becton-Dickinson, San Jose, CA).[13]

Results

Retrovirus vector

The structure of the retroviral vector used in this study is depicted in Figure 1. This vector carries two genes on MSCV backbone, one for Tm-specific cell growth and the other for *in vivo* tracking of transduced cells. The first cistron encodes DY703FGCRTmR, a modified chimeric receptor for Tm-responsive growth (Figure 1, upper). The prototype SAG encoded a fusion molecule (GCRER) between the full-length GCR and the estrogen binding domain (ER),[7] and we have modified this system with 3 major alterations. 1) The G-CSF binding domain of GCR was deleted (D).[7] This modification abrogated an undesired activation of the chimeric receptor by the endogenous G-CSF. 2) ER was replaced with a mutant HBD (TmR) which specifically binds to Tm, to circumvent limitations with wild-type ER.[10] In our previous study, up to 10^{-7} M of estrogen, which is 10- to 100-fold higher concentration than that of ovarian venous blood, had no influence on DGCRTmR-transduced Ba/F3 cells.[10] This modification greatly reduced nonspecific colony formation in murine progenitor assays (Ref. 10 and unpublished observation). 3) A tyrosine residue at position 703 in the murine GCR was mutated to phenylalanine (Y703F), to diminish differentiation signals.[14]

Downstream of the SAG, the IRES-controlled EGFP gene was linked for real-time identification of transduced cells. We showed that this type of vector was a versatile tool to track the progeny of transduced stem/progenitor cells.[13] *In vivo* proliferation and differentiation

of the transduced cells can be readily evaluated by multi-parameter flow cytometry at desired intervals.

For gene transfer to murine BM cells, transient viral supernatant was freshly prepared by transfecting BOSC23 cells with MSCV/DY703FGCRTmR-IRES-EGFP plasmid, and the titer of the viral supernatant was estimated to be 1 x 10^6 colony-forming units/mL.[13,16]

Retroviral transduction/reconstitution and Tm stimulation

In the present study, SAG-transduced cells were tracked in a murine bone marrow transplantation (BMT) model to investigate the impact of Tm stimulation. Ly5.2 BM cells were transduced by MSCV/DY703FGCRTmR-IRES-EGFP retrovirus following a fibronectin-assisted protocol.[13,17] After 48 hours of transduction, expression of EGFP in the BM cells was measured by flow cytometry. The frequencies of $EGFP^+$ cells ranged 5-20% upon 48 hours of transduction, depending on the retroviral infection cycles. In a representative experiment, the transduction efficiency was 20% (FACS data not shown), and the *ex vivo* transduced BM cells were transplanted. Lethally irradiated Ly5.1 mice were transplanted with MSCV/DY703FGCRTmR-IRES-EGFP-infected BM, 2 x 10^5 cells per animal. The transplants were individually numbered, and the peripheral blood (PB) was periodically drawn to monitor donor cell repopulation and EGFP expression. One month after BMT, donor cell chimerism was 82 ± 4% and $EGFP^+$ cells in Ly5.2 population were 20 ± 4% (Figure 2C). Two months post-BMT, 93 ± 2% of the host peripheral leukocytes were donor-derived (ie $Ly5.2^+$) and $EGFP^+$ cells were 21 ± 3%. At this point, the transplants were divided into 2 groups, one of which was challenged with Tm.

In our previous dose-response study using Ba/F3 cells, at least 10^{-7} M Tm was required to activate GCRTmR chimeric receptor.[10] To determine the Tm dosage for *in vivo* study, we gave

Tm to C57BL/6 mice with variable doses and regimens. Among them, 3 days of 1 mg Tm ip injection resulted 1-2 x 10^{-7} M in serum, and 3 days of 5 mg ip achieved 5-30 x 10^{-7} M (unpublished data). In the following Tm stimulation experiments, mice were given 5 mg/day Tm in 3 divided dose for 3 days.

Figure 2 shows the results of Tm administration to the primary BMT recipients. In the Tm-challenged group, every animal had an increased level of $EGFP^+$ cells in 2 weeks, and the escalation was statistically significant (21 ± 4% to 27 ± 5%, $p < 0.01$ by paired t-test ; Figure 2A). In contrast, the control animals had decreased percentages of $EGFP^+$ cells (21 ± 5% to 10 ± 3%, $p < 0.01$ by paired t-test; Figure 2B), and the $EGFP^+$ cells stayed at a lowered level (Figure 2C). The late-onset decrease of $EGFP^+$ cells in the controls was presumably because the long-term repopulating cells were less actively dividing than the short-term repopulating cells during *ex vivo* manipulation, therefore relatively refractory to retroviral transduction. On the other hand, an increase of $EGFP^+$ cells (27-28%) in the Tm-challenged mice was maintained for 1 month, then the transduced cells returned to the prestimulatory level and stabilized around 20% (Figure 2C). A significant difference of $EGFP^+$ leukocytes between the Tm-stimulated and the unstimulated groups was maintained for 3 months ($p < 0.05$ by Student's t-test), suggesting that MSCV/DY703FGCRTmR-IRES-EGFP-transduced hematopoietic progenitors proliferated more vigorously in response to Tm, that is, SAG-carrying cells expanded *in vivo*.

Tm challenge on the secondary recipients

Observing that Tm administration lead to a relative expansion of the gene-modified hematopoietic cells, we made secondary transplants to see whether the transducd long-term repopulating cells could be expanded by this drug. BM cells from unchallenged primary

recipients were pooled, and the aliquot cells were transplanted into irradiated Ly5.1 mice. A flow cytometry analysis showed that the frequency of $EGFP^+$ cells in the pooled BM was 21%, and 2×10^6 cells were infused into each animal (n = 8). One month following BMT, the donor cell chimerism was 65 ± 11% and the $EGFP^+$ cells were 19 ± 5% of $Ly5.2^+$ cells.

At this point, a subset of the secondary recipients (n = 4) was given Tm as the primary hosts were treated (5 mg ip for 3 days). Two weeks following Tm administration, $EGFP^+$ cells showed a significant increase (16 ± 4% to 35 ± 3%, $p < 0.02$ by paired t-test; Figure 3A), in contrast to a decrease in the control animals (22 ± 4% to 12 ± 4%, $p < 0.01$ by paired t-test; Figure 3B). Subsequently, $EGFP^+$ cells in the stimulated animals returned to the prestimulatory level and stabilized at 14-18% (Figure 3C). On the other hand, transduced cells in the unstimulated animals further decreased, and the difference between the two groups was statistically significant for more than 3 months (Figure 3C). These results indicated that very immature cells, early progenitors or stem cells, were tansduced with the SAG and maintained for at least 6 months through serial transplantation. In the presence of Tm, the transduced cells appeared to have proliferative advantage over untransduced cells, therefore gave rise to more EGFP+ leukocytes in the stimulated animals. The most active expansion was likely to occur at a late progenitor level since an overt response was observed in a few weeks and subsequently subsided. In addition, the fact that Tm stimulation lead to a relative but prolonged elevation of transduced cells suggested that Tm-responsive expansion occurred at more immature stages as well.

Lineage analysis

As expected from the relatively short-term increase of $EGFP^+$ leukocytes upon Tm administration,

the responding cells were mostly derived from myeloid progenitors. To determine how each hematopoietic lineage responded to Tm, the secondary transplants were subjected to a detailed FACS analysis 4 months following BMT (ie 3 months after Tm stimulation). The transplant PB cells were gated for granulocytes ($Gr1^{high}/Mac1^{+}$), monocytes ($Gr1^{low-mid}/Mac1^{+}$), $CD4^{+}$ T cells ($CD4^{+}/CD8^{-}$), $CD8^{+}$ T cells ($CD4^{-}/CD8^{+}$), B cells ($IgM^{+}/B220^{+}$) and NK cells ($NK1.1^{+}/TCRb^{-}$), and $EGFP^{+}$ cells were enumerated in each subset (Figure 4). In the control animals, granulocytes contained slightly higher percentages of $EGFP^{+}$ cells (7 ± 5%) than other leukocyte subsets (monocytes 2 ± 1%, $CD4^{+}$ T cells 1 ± 1%, $CD8^{+}$ T cells 1 ± 1%, B cells 1 ± 1%, and NK cells 4 ± 2%). This predisposition was eminent in the Tm-challenged mice. These animals had significantly higher percentages of $EGFP^{+}$ granulocytes (51 ± 31%) than other cell types (monocytes 7 ± 4%, $CD4^{+}$ T cells 1 ± 1%, $CD8^{+}$ T cells 2 ± 1%, B cells 1 ± 0%, and NK cells 6 ± 2%; $p < 0.05$ by Student's t-test). As for granulocytes and monocytes, $EGFP^{+}$ frequency between the challenged mice and the controls was also significant (51 ± 31% vs. 7 ± 5% in granulocytes, and 7 ± 4% vs. 2 ± 1% in monocytes; $p < 0.05$ by Student's t-test). These results suggested that the DY703FGCRTmR-mediated growth signal worked most efficiently in myeloid progenitors, particularly granulocyte precursors.

Discussion

In this study, we harnessed mouse BM cells with a modified SAG and examined whether they had an *in vivo* growth advantage under specific stimulation. The SAG was designed for a GCR-based chimeric molecule to generate a growth signal in response to Tm, with minimal myeloid differentiation. After hematopoietic reconstitution with the SAG-transduced BM, Tm

administration resulted in an increase of transduced cells. Moreover, Tm stimulation lead to an increase of the transduced cells after secondary reconstitution, indicating that long-term repopulating cells can be expanded. However, the escalation of gene-modified cells was relatively short-term and the transduced cell level returned to the prestimulatory level. This result suggested that a majority of expanded cells upon Tm stimulation was in the late progenitor compartment with definite proliferative capacity. Still, some SAG-transduced long-term repopulating cells appeared to maintain $EGFP^+$ cells at a higher level than that in the control animals.

The idea of *in vivo* cell expansion by conferring a direct growth advantage has been reported by others, too. Blau et al have developed a similar chimeric receptor system in which FKBP-based fusion proteins are cross-linked with dimeric compounds. With a fusion of Mpl (thrombopoietin receptor) cytoplasmic domain and FKBP, they expanded murine hematopoietic cells by giving a synthetic dimerizer molecule AP20187.[19] It is of note that their expansion was also transient, suggesting that major responders were analogous cells we postulated in our study.

The stage specificity of responding cells may account for the lineage specificity that was shown in the results presented. Although Y703F mutation diminished a granulocyte differentiation signal in 32D cells,[14] *in vivo* stimulation of DY703FGCRTmR-transduced cells resulted in a preferential expansion of granulocytes. We speculate that in a homogeneous cell population such as 32D cell line, the effect of abolishing differentiation signal was readily appreciated. In a heterogeneous population such as hematopoietic reconstituting cells, however, the readout of expansion may be greatly influenced by differential responsiveness of the cells in different lineages. If the GCR-mediated growth signal works most efficiently in the already committed late myeloid progenitors, enhancing cell cycling in this compartment would