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ARTICLE

Total body irradiation must be delivered at high dose for efficient engraftment and tolerance in a rhesus stem cell gene therapy model

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Reduced intensity conditioning (RIC) is desirable for hematopoietic stem cell (HSC) gene therapy applications. However, low gene marking was previously observed in gene therapy trials, suggesting that RIC might be insufficient for (i) opening niches for efficient engraftment and/or (ii) inducing immunological tolerance for transgene-encoded proteins. Therefore, we evaluated both engraftment and tolerance for gene-modified cells using our rhesus HSC gene therapy model following RIC. We investigated a dose de-escalation of total body irradiation (TBI) from our standard dose of 10Gy (10, 8, 6, and 4Gy), in which rhesus CD34$^+$ cells were transduced with a VSVG-pseudotyped chimeric HIV-1 vector encoding enhanced green fluorescent protein (GFP) (or enhanced yellow fluorescent protein (YFP)). At ~6 months after transplantation, higher-dose TBI resulted in higher gene marking with logarithmic regression in peripheral blood cells. We then evaluated immunological tolerance for gene-modified cells, and found that lower-dose TBI allowed vigorous anti-GFP antibody production with logarithmic regression, while no significant anti-VSVG antibody formation was observed among all TBI groups. These data suggest that higher-dose TBI improves both engraftment and immunological tolerance for gene-modified cells. Additional immunosuppression might be required in RIC to induce tolerance for transgene products. Our findings should be valuable for developing conditioning regimens for HSC gene therapy applications.

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INTRODUCTION

Hematopoietic stem cell (HSC)-targeted gene therapy is potentially curative for various inherited diseases, and several groups have recently reported therapeutic benefit in gene therapy trials.[1–6] Reduced intensity conditioning (RIC) regimens in the autologous setting are desirable for HSC-targeted gene therapy, as immunologic barriers encountered in the allogeneic HSC transplantation setting are not present and the perceived need for immunosuppression is not required. In addition, conventional myeloablative conditioning may not be well tolerated for many desired applications, including severe immunodeficiency due to chronic infections and sickle cell disease (SCD) due to end organ damage. However, in previous gene therapy trials, low gene marking was reported in peripheral blood cells,[1–6] raising the question that RIC might be insufficient for efficient engraftment of gene-modified HSCs expressing neoantigens.

Conditioning regimens are designed to promote hematopoietic stem cell engraftment by the cells administered over those cells that reside in the marrow space. The conditioning therapy contains two major effects: (i) myelosuppression to open the niche space for HSC engraftment; and (ii) immunosuppression to prevent graft rejection.[7] Traditionally, high-intensity myeloablative conditioning regimens have been used for allogenic transplantation applications, which carry a high risk of treatment related morbidity, and to a lesser extent, mortality. As such, high-intensity conditioning is less suitable for patients with potential and/or existing organ damage, including elder patients and adult SCD patients. In addition, conventional myeloablative conditioning is important to reduce/eliminate residual leukemia or lymphoma cells in patients with hematological malignancies; however, this additional goal is not relevant to gene therapy for nonmalignant diseases.[8] Recently, RIC regimens were developed to improve transplant-related mortality, and results in adult SCD patients demonstrate acceptable safety parameters.[9,10]

We previously established an HSC-targeted gene therapy model in rhesus macaques with high gene marking in peripheral blood cells long-term after transplantation following a myeloablative dose of 10Gy total body irradiation (TBI).[11,12] This large animal model allows us to evaluate efficiency for gene marking in hematopoietic repopulating cells, immunological reactions to gene-modified cells, and safety (insertional mutagenesis) in various gene therapy settings during extended follow-up.[13–15] Using our rhesus gene therapy model, in the current study, we

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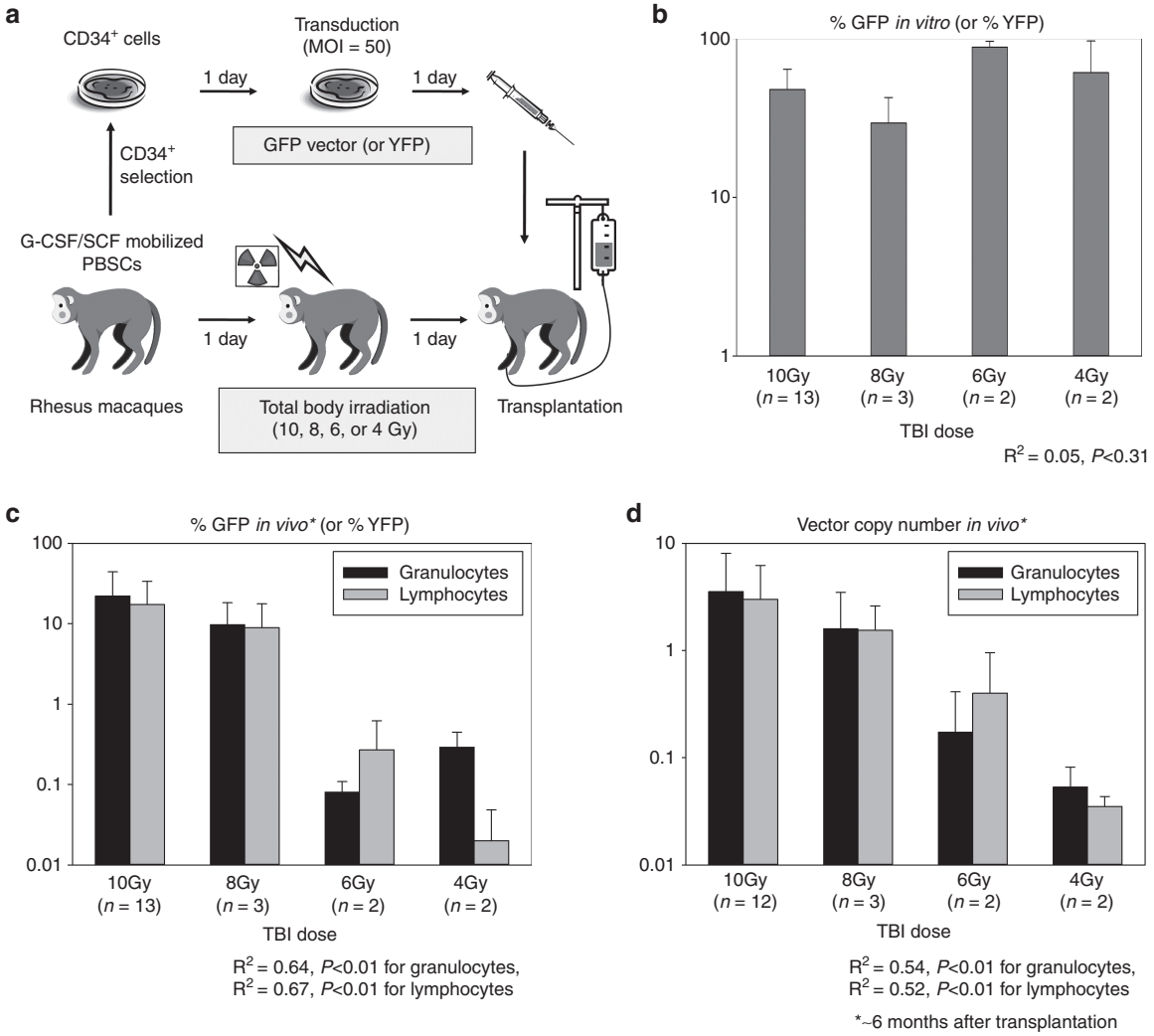

Figure 1 Increasing doses of total body irradiation (TBI) results in higher marking levels *in vivo* in a rhesus gene therapy model. (**a**) To evaluate whether reduced intensity conditioning (RIC) is sufficient for hematopoietic stem cell-targeted gene therapy, we performed dose de-escalation of TBI (10, 8, 6, and 4Gy) in a rhesus gene therapy model. Granulocyte-colony stimulating factor (G-CSF) and stem cell factor (SCF)-mobilized rhesus CD34$^+$ cells were transduced with an enhanced green fluorescent protein (GFP) (or enhanced yellow fluorescent protein (YFP))-expressing lentiviral vector at multiplicity of infection (MOI) 50, and the transduced CD34$^+$ cells were infused into rhesus macaques following various doses of TBI conditioning. At ~6 months after transplantation, we evaluated gene marking levels in granulocytes and lymphocytes, which were analyzed by GFP (or YFP) positive rates (%GFP) and vector copy numbers (VCNs). (**b**) A small aliquot of transduced CD34$^+$ cells were cultured *in vitro* to evaluate transduction efficiency. We observed efficient transduction (%GFP) for transduced rhesus CD34$^+$ cells *in vitro* among all TBI groups. (**c**) Increasing doses of TBI resulted in higher %GFP with logarithmic regression in both granulocytes and lymphocytes. (**d**) Similar positive logarithmic correlation was observed between TBI doses and VCNs in both granulocytes and lymphocytes. These data suggest that higher doses of TBI improve engraftment of gene-modified hematopoietic repopulating cells.

investigated dose de-escalation of TBI as means to evaluate RIC transplantation, and evaluated both engraftment and tolerance for gene-modified cells.

RESULTS

Increasing doses of TBI result in higher marking levels *in vivo* in a rhesus gene therapy model

To evaluate whether RIC is insufficient for (i) opening niches for efficient engraftment and (ii) inducing immunological tolerance for transgenes, we evaluated both gene marking levels and immunological response in 20 rhesus macaques who received a dose de-escalation of TBI (10, 8, 6, and 4Gy) in an HSC-targeted gene therapy model (Figure 1a). The 10Gy TBI was previously established in our rhesus macaque model and allows stable, high-level gene marking *in vivo* years after transplantation, while in the current study, decreasing doses of TBI (8-4Gy) were used as RIC transplantation.[11–13,15] The mobilized rhesus CD34$^+$ cells were transduced with a vesicular stomatitis virus glycoprotein (VSVG)-pseudotyped chimeric HIV-1 vector encoding enhanced green fluorescent protein (GFP) (or YFP), which allows us to efficiently transduce rhesus hematopoietic cells as well as human hematopoietic cells.[11,12,16] The transduced CD34$^+$ cells were transplanted into autologous animals following 10-4Gy of TBI conditioning. We evaluated gene marking levels *in vivo* in peripheral blood ~6 months after transplantation, by quantifying the fraction of GFP (or YFP)-positive cells (%GFP) by flow cytometry and average vector copy number per cell (VCN) by real-time polymerase chain reaction.

To confirm whether rhesus CD34$^+$ cells were sufficiently transduced with lentiviral vectors, we cultured a small aliquot of transduced CD34$^+$ cells and evaluated *in vitro* %GFP 3–4 days after

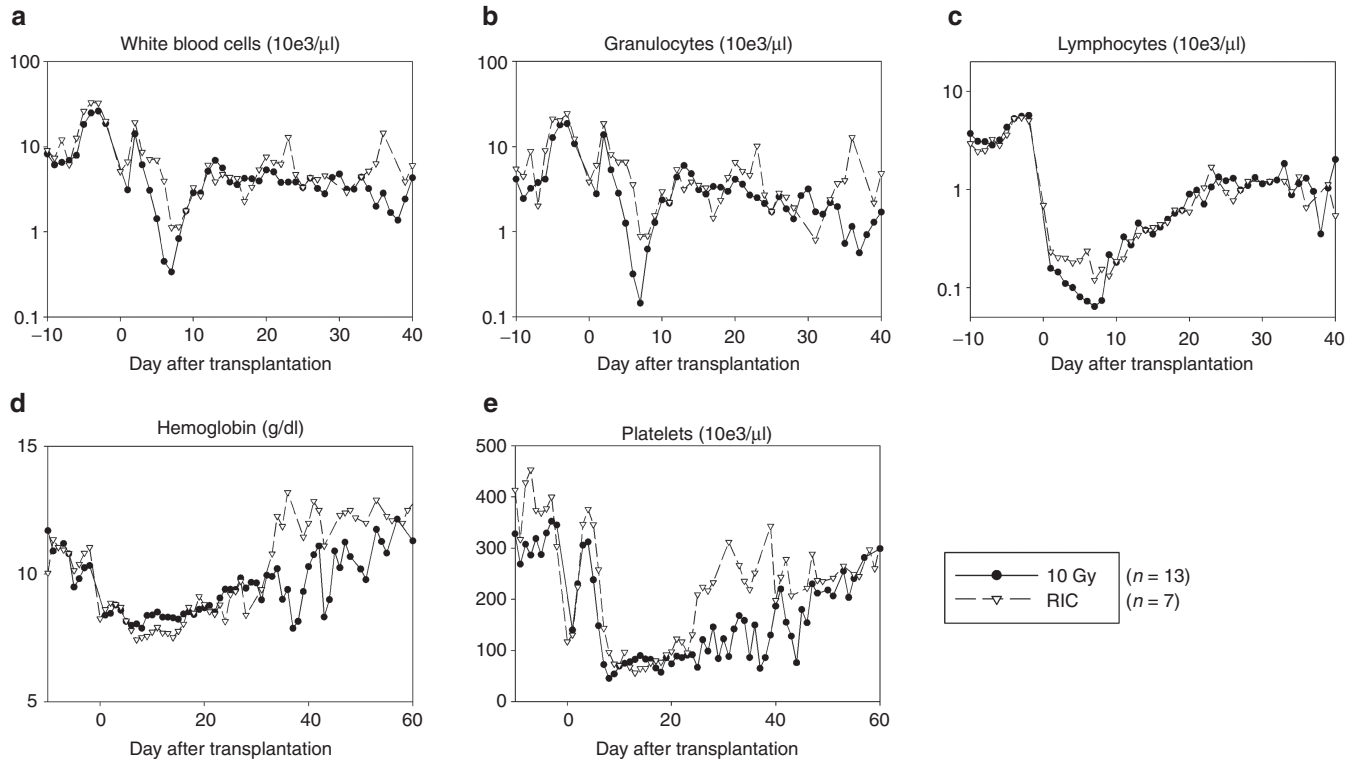

Figure 2 The blood cell counts were more mildly and slowly reduced in the reduced intensity conditioning (RIC) animals. (**a,b,c**) The milder and slower reduction of blood counts was observed in white blood cells, granulocytes, and lymphocytes among the RIC animals (8-4Gy total body irradiation (TBI)), as compared to 10Gy TBI. (**d** and **e**) We observed relatively faster recovery of hemoglobin concentrations and platelet counts after RIC transplantation.

transduction. Efficient transduction (22–71%) was observed for rhesus CD34$^+$ cells among all TBI groups, and there was no correlation between TBI doses and *in vitro* %GFP ($R^2 = 0.05$, $P = 0.31$) (Figure 1b). After RIC (8-4Gy TBI) and transplantation of transduced CD34$^+$ cells, reductions in blood cell counts progressed more slowly in white blood cells, granulocytes, and lymphocytes, as compared to 10Gy TBI (Figure 2a,b,c). Predictably, we also observed a relatively faster recovery of hemoglobin concentrations and platelet counts in the RIC animals (Figure 2d,e). GFP-positive cells were initially observed in peripheral blood cells among all animals. In the animals receiving a myeloablative dose of 10Gy TBI conditioning, intermediate %GFP (22–25%) and VCNs (3.0–3.6) were observed in peripheral blood cells ~6 months after transplantation (Figure 1c,d). Increasing doses of TBI resulted in higher %GFP with logarithmic regression in both granulocytes ($R^2 = 0.64$, $P < 0.01$) and lymphocytes ($R^2 = 0.67$, $P < 0.01$) (Figure 1c). A similar positive correlation was observed between TBI doses and VCNs in both granulocytes ($R^2 = 0.54$, $P < 0.01$) and lymphocytes ($R^2 = 0.52$, $P < 0.01$) (Figure 1d). These data demonstrate that higher doses of TBI improve engraftment of gene-modified hematopoietic repopulating cells.

Lower doses of TBI allow anti-GFP antibody production in a rhesus gene therapy model

At lower doses of TBI, we initially observed very high %GFP (89–97%) confined to the granulocyte fraction of peripheral blood cells at early time points (1–3 months) after transplantation, as compared to the lymphocyte fraction ($P < 0.05$ in 6-4Gy at ~3 months post-transplant) (Figure 3), suggesting an immunological response against GFP and subsequent phagocytosis.[13] To evaluate

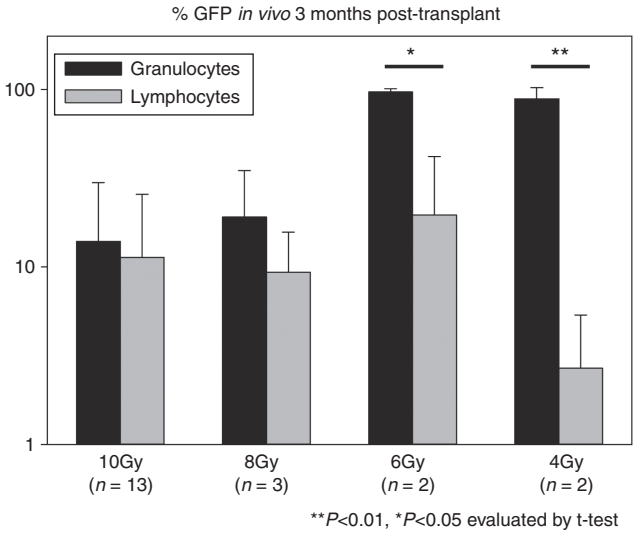

Figure 3 Very high %GFP was initially observed in granulocytes ~3 months after lower-dose total body irradiation (TBI) conditioning. In lower doses of TBI conditioning animals (6-4Gy), we initially observed very high %GFP (close to 100%) specific for the granulocyte fraction of peripheral blood cells at ~3 months after transplantation (not in the lymphocyte fraction), suggesting immunological responses to GFP and subsequent phagocytosis.

immunological tolerance for gene-modified cells, we measured both anti-GFP and anti-VSVG antibody titers using serum samples obtained ~6 months after transplantation. The animals receiving myeloablative conditioning of 10Gy TBI did not produce either

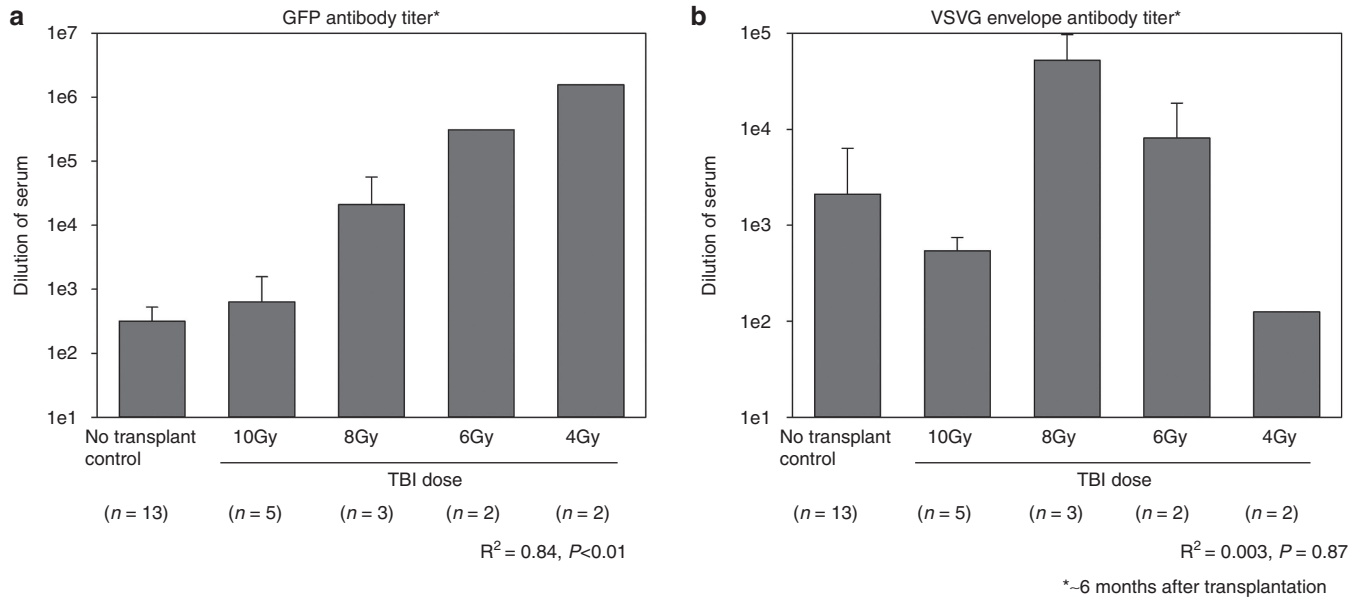

Figure 4 Lower doses of total body irradiation (TBI) induces anti-GFP antibody production. (**a**) To evaluate immunological tolerance for gene-modified cells, we measured both anti-GFP and anti-vesicular stomatitis virus glycoprotein (VSVG) antibody titers in the transplanted rhesus macaques. Lower-dose TBI allowed anti-GFP antibody production with logarithmic regression. (**b**) No such effect on anti-VSVG antibody was observed among all TBI groups. The background levels in no antigen-coated controls were 1e2 for the anti-GFP antibody and undetectable for the anti-VSVG antibody. These data demonstrate that higher dose TBI is important to induce immunological tolerance for gene-modified cells in a rhesus transplantation model.

anti-GFP or anti-VSVG antibodies after transplantation (Figure 4a,b). However, RIC transplantation at lower-dose TBI allowed for high-titer anti-GFP antibody production with logarithmic regression (R^2 = 0.84, P < 0.01) (Figure 4a). No such effect on anti-VSVG antibody production was observed among all TBI groups (R^2 = 0.003, P = 0.87) (Figure 4b). The background levels in no antigen-coated controls were detectable in only the lowest dilution of serum (1e2) for the anti-GFP antibody and undetectable for the anti-VSVG antibody. These data demonstrate that higher-dose TBI is important to induce immunological tolerance for transgene products in gene-modified cells in our rhesus gene therapy model.

DISCUSSION

In this study, we investigated de-escalating doses of TBI conditioning in our rhesus gene therapy model with lentiviral transduction, and demonstrated that high-dose TBI (10Gy) is important for both more efficient engraftment of gene-modified cells and induction of immunological tolerance for the transgene product (GFP). Previously, we demonstrated that immunologic tolerance can be induced at 10Gy TBI[17] and that at lower doses of TBI, residual $CD4^+$ cells remain in immune tissues.[15] Utilizing RIC doses of TBI (8-4Gy), lower gene marking levels and high anti-GFP antibody production were observed with regression in the transplanted rhesus macaques.

In developing conditioning therapies for HSC transplantation, myelosuppression is thought to be important to open stem cell niches in the bone marrow.[7] According to our current understanding, HSCs (and primitive progenitor cells) can attach to the stem cell niche, and through this attachment, HSCs maintain their properties of self-renewal and multipotency.[18] Once HSCs are released from the niche, HSCs start differentiation and progressively lose these features. Following RIC transplantation for gene therapy application, the gene-modified HSCs must therefore compete with remaining host HSCs (native, unmodified HSCs, which presumably retain their attachment to the HSC niche) in order to gain access to open niche for engraftment, much like musical chairs. In allogeneic HSC transplantation, the donor lymphocytes contained within or deriving from the graft can remove the remaining recipient HSCs from their niche through immunological mechanisms permitting donor HSC engraftment, which can be enhanced further by donor lymphocyte infusions.[7] This could in part explain the much higher level of myeloid engraftment (mean donor chimerism at 3.6 years of 86%) seen in our recipients of allogeneic HSC transplantation after low-dose TBI at 3Gy in conjunction with alemtuzumab and sirolimus in adults with SCD.[9] However, this mechanism for enhanced engraftment does not occur in the gene therapy setting, and both gene-modified HSCs and remaining unmodified host HSCs can remain stably in the niches, since autologous HSCs are used for gene modification and the gene-modified graft cells should immunologically recognize the remaining host HSCs as self. Additionally, whether lower-dose TBI, such as that used in our allogeneic clinical trials for SCD, would be sufficient for obtaining high-level engraftment if the immunologic barrier were overcome has not been answered in the current study. We observed consistently in our rhesus gene therapy model that higher doses of TBI resulted in more efficient engraftment of gene-modified cells. Therefore, sufficient myelosuppression to open HSC niches appears paradoxically more important for efficient engraftment in autologous gene-modified HSC transplantation, as compared to allogeneic HSC transplantation.

In allogeneic HSC transplantation, immunosuppression is crucial to prevent immunological rejection, and additional immunosuppressive therapies are generally used to also reduce the risk of graft versus host disease.[7,8] In the gene therapy setting, immunosuppression has long been deemed unnecessary, since autologous HSCs are used for gene modification (lentiviral transduction). This certainly held true for the immunodeficiencies which were initially targeted for gene therapy applications.[6] However, we hypothesized that therapeutic transgene products and/or lentiviral components could induce immunological rejection from remaining host lymphocytes to gene-modified graft derived cells. In our rhesus gene therapy

model, 10Gy TBI resulted in efficient engraftment with no antibody production to either GFP or the VSVG envelope, while lower doses of TBI (8-4Gy) indeed permitted high-titer anti-GFP antibody production at 6 and 4Gy, suggesting immunological rejection. At 8Gy TBI, long-term engraftment was observed. These data suggest that additional immunosuppression may be helpful to induce immunological tolerance for transgene products (GFP) to allow RIC in gene therapy applications targeting HSCs. On the other hand, gene therapy trials utilizing vectors that encode a protein to which the patient is already tolerant may not require such additional immunosuppression as we observed no immunologic reaction to viral vector components.

The availability of an intravenous formulation of the long-utilized alkylating agent, busulfan, has made this drug an attractive agent for a RIC regimen for gene therapy.[1,2,5] In recent gene therapy trials for β-thalassemia and SCD,[3] a myeloablative dose of busulfan conditioning is used, and currently these trials are ongoing at several institutions. While TBI conditioning regimens are effective for both myelosuppression and immunosuppression, busulfan conditioning is predominantly myelosuppressive, with a relative sparing of the immune system. Theoretically, a myeloablative dose of busulfan conditioning should be sufficient to open niches, resulting in efficient engraftment of gene-modified cells, but this leaves the concern that additional immunosuppression should be added to the conditioning to induce immunological tolerance against a therapeutic β-globin transgene. On the other hand, SCD patients encode similar proteins, including sickle globin and δ-globin, which may confer immunologic tolerance for a therapeutic β-globin protein without additional immunosuppression. We have previously demonstrated that busulfan produces dose-dependent engraftment in the murine setting with a prolonged window for HSC infusion.[19] Furthermore, busulfan pharmacokinetics is predictable in the rhesus.[20,21] We have now begun further experiments to investigate whether additional immunosuppression will permit immunological tolerance for transgene products including highly immunogenic proteins such as GFP or proteins to which the animals are presumably tolerant such as β-globin or γ-globin following busulfan conditioning in our rhesus gene therapy model.

After lower doses of TBI conditioning (6-4Gy), we observed very high %GFP (92.8±9.4%) in the granulocyte fraction at ~3 months after transplantation. However, these GFP-positive cells had low VCNs (0.10±0.14) and VCNs declined to undetectable levels (<1%) at ~6 months. These data suggest that the GFP is not vector-encoded in these cells, but that the GFP protein is phagocytized by these cells in the granulocyte fraction ~3 months after transplantation. We previously observed strong T-lymphocyte reactions to GFP-positive cells *in vitro*, in rhesus RIC transplantation,[13] suggesting that both humoral and cellular immune responses are involved in the GFP reaction.

In summary, higher doses of TBI increased gene-marking levels in transplanted rhesus macaques, while lower doses of TBI permitted anti-GFP antibody production leading to graft rejection. Additional immunosuppressive therapy might be required in RIC to induce immunological tolerance for transgene products. Our findings should be valuable to consider conditioning regimens for clinical gene therapy.

MATERIALS AND METHODS

Rhesus HSC-targeted gene therapy model with TBI dose de-escalation

We previously developed a chimeric HIV-1-based lentiviral vector (χHIV vector) system, in which the HIV-1 genomes were packaged into the simian immunodeficiency virus capsid to efficiently transduce rhesus hematopoietic cells as well as human cells.[11,12,16] Granulocyte colony-stimulating factor (Amgen, Thousand Oaks, CA) and stem cell factor (Amgen)-mobilized rhesus $CD34^+$ cells were transduced with VSVG-pseudotyped χHIV vectors encoding GFP (or YFP) at multiplicity of infection (MOI) of 50, and the transduced cells were transplanted into rhesus macaques following a dose de-escalation of TBI (10, 8, 6, and 4Gy).[11–13,15] The TBI conditioning was performed over two equal doses (2x5Gy, 2x4Gy, and 2x3Gy) except for 4Gy TBI (1x4Gy). The 10Gy TBI was known as a myeloablative dose, while the TBI doses decreased as RIC regimens (8-4Gy).

At ~6 months after transplantation, we evaluated both %GFP in peripheral blood cells by flow cytometry (FACSCalibur; BD Biosciences, Franklin Lakes, NJ), and the VCNs by real-time polymerase chain reaction, as previously described.[11,22] For the statistical analysis, animals transplanted in competitive repopulation assays were scored by doubling the %GFP and VCNs derived from the half of $CD34^+$ cells transduced with the χHIV vector.

Evaluation of anti-GFP or anti-VSVG antibody production by enzyme-linked immunosorbent assay (ELISA)

We performed ELISA to quantitate anti-GFP or anti-VSVG antibodies in the rhesus serum ~6 months (2–10 months) after transplantation, as previously described.[13] Briefly, we coated a 96-well plate with a recombinant GFP protein (Clontech, Mountain View, CA) or a VSVG envelope protein produced in transfected 293T cells, and added serial dilutions of monkey serum into these wells. After incubation and washing, anti-monkey IgG secondary antibody conjugated to horseradish peroxidase (Abcam, Cambridge, MA) was added to detect GFP- or VSVG-bound immunoglobulin, and tetramethylbenzidine reaction signals were detected by absorbance at 450 nm.

Statistical analysis

Statistical analyses were performed using the JMP 11 software (SAS Institute, Cary, NC). The averages in various conditions were evaluated by Dunnett's test (one-way analysis of variance for a control). Two averages were evaluated by the student's *t*-test. Correlations were evaluated by R square in logarithmic regression and p values in correlation coefficient. A P value of <0.01 or <0.05 was deemed significant. Standard deviations were shown as error bars in all figures.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1. Aiuti, A, Cattaneo, F, Galimberti, S, Benninghoff, U, Cassani, B, Callegaro, L *et al.* (2009). Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N Engl J Med* **360**: 447–458.
2. Aiuti, A, Slavin, S, Aker, M, Ficara, F, Deola, S, Mortellaro, A *et al.* (2002). Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning. *Science* **296**: 2410–2413.
3. Cavazzana-Calvo, M, Payen, E, Negre, O, Wang, G, Hehir, K, Fusil, F *et al.* (2010). Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. *Nature* **467**: 318–322.
4. Hacein-Bey-Abina, S, von Kalle, C, Schmidt, M, Le Deist, F, Wulffraat, N, McIntyre, E *et al.* (2003). A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* **348**: 255–256.
5. Ott, MG, Schmidt, M, Schwarzwaelder, K, Stein, S, Siler, U, Koehl, U *et al.* (2006). Correction of X-linked chronic granulomatous disease by gene therapy, augmented by insertional activation of MDS1-EVI1, PRDM16 or SETBP1. *Nat Med* **12**: 401–409.
6. Schwarzwaelder, K, Howe, SJ, Schmidt, M, Brugman, MH, Deichmann, A, Glimm, H *et al.* (2007). Gammaretrovirus-mediated correction of SCID-X1 is associated with skewed vector integration site distribution in vivo. *J Clin Invest* **117**: 2241–2249.

7. Copelan, EA (2006). Hematopoietic stem-cell transplantation. *N Engl J Med* **354**: 1813–1826.
8. Gyurkocza, B and Sandmaier, BM (2014). Conditioning regimens for hematopoietic cell transplantation: one size does not fit all. *Blood* **124**: 344–353.
9. Hsieh, MM, Fitzhugh, CD, Weitzel, RP, Link, ME, Coles, WA, Zhao, X *et al.* (2014). Nonmyeloablative HLA-matched sibling allogeneic hematopoietic stem cell transplantation for severe sickle cell phenotype. *JAMA* **312**: 48–56.
10. Hsieh, MM, Kang, EM, Fitzhugh, CD, Link, MB, Bolan, CD, Kurlander, R *et al.* (2009). Allogeneic hematopoietic stem-cell transplantation for sickle cell disease. *N Engl J Med* **361**: 2309–2317.
11. Uchida, N, Hargrove, PW, Lap, CJ, Evans, ME, Phang, O, Bonifacino, AC *et al.* (2012). High-efficiency transduction of rhesus hematopoietic repopulating cells by a modified HIV1-based lentiviral vector. *Mol Ther* **20**: 1882–1892.
12. Uchida, N, Washington, KN, Hayakawa, J, Hsieh, MM, Bonifacino, AC, Krouse, AE *et al.* (2009). Development of a human immunodeficiency virus type 1-based lentiviral vector that allows efficient transduction of both human and rhesus blood cells. *J Virol* **83**: 9854–9862.
13. Uchida, N, Weitzel, RP, Evans, ME, Green, R, Bonifacino, AC, Krouse, AE *et al.* (2014). Evaluation of engraftment and immunological tolerance after reduced intensity conditioning in a rhesus hematopoietic stem cell gene therapy model. *Gene Ther* **21**: 148–157.
14. Uchida, N, Bonifacino, A, Krouse, AE, Metzger, ME, Csako, G, Lee-Stroka, A *et al.* (2011). Accelerated lymphocyte reconstitution and long-term recovery after transplantation of lentiviral-transduced rhesus CD34+ cells mobilized by G-CSF and plerixafor. *Exp Hematol* **39**: 795–805.
15. Donahue, RE, Srinivasula, S, Uchida, N, Kim, I, St Claire, A, Duralde, G *et al.* (2015). Discordance in lymphoid tissue recovery following stem cell transplantation in rhesus macaques: an *in vivo* imaging study. *Blood* **126**: 2632–2641.
16. Uchida, N, Hsieh, MM, Washington, KN and Tisdale, JF (2013). Efficient transduction of human hematopoietic repopulating cells with a chimeric HIV1-based vector including SIV capsid. *Exp Hematol* **41**: 779–788.e1.
17. Kung, SK, An, DS, Bonifacino, A, Metzger, ME, Ringpis, GE, Mao, SH *et al.* (2003). Induction of transgene-specific immunological tolerance in myeloablated nonhuman primates using lentivirally transduced CD34+ progenitor cells. *Mol Ther* **8**: 981–991.
18. Moore, KA and Lemischka, IR (2006). Stem cells and their niches. *Science* **311**: 1880–1885.
19. Hsieh, MM, Langemeijer, S, Wynter, A, Phang, OA, Kang, EM and Tisdale, JF (2007). Low-dose parenteral busulfan provides an extended window for the infusion of hematopoietic stem cells in murine hosts. *Exp Hematol* **35**: 1415–1420.
20. Kang, EM, Hsieh, MM, Metzger, M, Krouse, A, Donahue, RE, Sadelain, M *et al.* (2006). Busulfan pharmacokinetics, toxicity, and low-dose conditioning for autologous transplantation of genetically modified hematopoietic stem cells in the rhesus macaque model. *Exp Hematol* **34**: 132–139.
21. Kahl, CA, Tarantal, AF, Lee, CI, Jimenez, DF, Choi, C, Pepper, K *et al.* (2006). Effects of busulfan dose escalation on engraftment of infant rhesus monkey hematopoietic stem cells after gene marking by a lentiviral vector. *Exp Hematol* **34**: 369–381.
22. Uchida, N, Evans, ME, Hsieh, MM, Bonifacino, AC, Krouse, AE, Metzger, ME *et al.* (2013). Integration-specific *In Vitro* Evaluation of Lentivirally Transduced Rhesus CD34(+) Cells Correlates With *In Vivo* Vector Copy Number. *Mol Ther Nucleic Acids* **2**: e122.