Published in final edited form as:

J Immunother. 2011 May ; 34(4): 343–352. doi:10.1097/CJI.0b013e3182187600.

Genetic engineering of murine CD8⁺ and CD4⁺ T cells for pre-clinical adoptive immunotherapy studies

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Abstract

T-cell-receptor (TCR) gene therapy enables for the rapid creation of antigen-specific T cells from mice of any strain and represents a valuable tool for pre-clinical immunotherapy studies. Here, we describe the superiority of gamma-retroviral vectors compared to lentiviral vectors for transduction of murine T cells and surprisingly illustrate robust gene-transfer into phenotypically naïve/memory-stem cell (CD62L^{hi}/CD44^{low}) and central memory (CD62L^{hi}/CD44^{hi}) CD8⁺ T cells using murine-stem-cell-based gamma-retroviral vectors (MSGV1). We created MSGV1 vectors for a MHC-class I restricted T-cell receptor (TCR) specific for the melanocyte-differentiation antigen, gp100 (MSGV1-pmel-1), and a MHC-class II restricted TCR specific for tyrosinase-related-protein-1 (MSGV1-TRP-1), and found that robust gene expression required codon optimization of TCR sequences for the pmel-1 TCR. To test for functionality, we adoptively transferred TCR-engineered T cells into mice bearing B16 melanomas and observed delayed growth of established tumors with pmel-1TCR engineered CD8⁺ T cells and significant tumor regression with TRP-1 TCR transduced CD4⁺ T cells. We simultaneously created lentiviral vectors encoding the pmel-1TCR, but found that these vectors mediated low TCR expression in murine T cells, but robust gene expression in other murine and human cell lines. These results indicate that preclinical murine models of adoptive immunotherapies are more practical using gamma-retroviral rather than lentiviral vectors.

INTRODUCTION

The adoptive transfer of T cells engineered to express a tumor specific T-cell receptor (TCR) represents a promising treatment for patients with metastatic cancer ^{1–10}. To improve the results of current TCR gene therapies, preclinical models need to be established to rapidly test biological concepts that may advance existing treatment regimens. Gamma-retroviral and lentiviral vectors are capable of mediating stable gene transfer into mammalian cells ^{11–16}. However, post-thymic murine T cells represent a unique population of cells central to a host's adaptive immune arm and generating optimal protocols for mediating gene transfer into these cells *ex vivo* would be valuable for researchers seeking to rapidly create antigen-specific T cells on different genetic background strains ¹⁷.

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The authors declare that there are no potential conflicts of interests

Established murine tumor models of adoptive transfer utilize γ -retroviral mediated gene transfer of TCRs into actively dividing murine T cells^{6, 18, 19}. However, less differentiated CD8⁺ T cells may represent the ideal population of cells to mediate tumor regression and recent evidence suggests that lentiviral based vectors can mediate stable gene transfer into non-dividing naïve/memory-stem cell (T_{N/SCM}) and central memory (T_{CM}) T cells^{20–26}. Another potential advantage of lentiviral vectors is the low propensity to integrate into the promoter regions of transcribed genes, which may be associated with insertional oncogenesis²⁷. This has led to the rapid development of clinical grade self-inactivating lentiviral vectors for human gene therapy trials^{28–31} and we therefore attempted to develop a practical preclinical model to assist in directing future clinical endeavors utilizing both γ -retroviral and lentiviral vectors.

Contrary to our initial hypothesis, we found that γ -retroviral vectors mediated efficient gene delivery into T_N and T_{CM} cells after a short-term (24 hour) anti-CD3/anti-CD28 stimulation. In comparison, lentiviral vectors mediated poor gene transfer specifically into murine T cells while robustly delivering genes into murine fibroblasts, human jurkat cells, and human CD4⁺ and CD8⁺ T cells. Developing effective models for HIV infection in murine T cells remains a challenge, but would represent a valuable tool for researchers across multiple disciplines.

MATERIALS AND METHODS

Mice and Cell Lines

Splenocytes from C57BL/6 and CD8^{−/−} mice (Jackson Laboratories) were used to generate CD8⁺ and CD4⁺ murine T cells, respectively. Murine 3T3 and human jurkat-T3 cells (ATCC, TIB-152) were maintained in culture as previously described. Human peripheral blood lymphocytes were obtained from patients with metastatic melanoma seeking treatment at the Surgery Branch, National Cancer Institute. Murine splenocytes were stimulated with either 1 μ g/mL of anti-CD3 and anti-CD28 (BD Biosciences, San Jose, CA) or con A (2 μ g/mL) and IL-7 (1 ng/mL; Peprotech, Rocky Hill, NJ) for 24 or 48 hours and cultured in 60 IU/mL of recombinant human IL-2 (Chiron, Emeryville, CA) IL-15 (10 ng/mL), or IL-21 (10 ng/mL; Peprotech, Rocky Hill, NJ). Complete media consisted of either DMEM high glucose for viral producer cell lines or RPMI for T cells (Invitrogen, Carlsbad, CA) mixed with 10% Fetal Bovine Serum, 1% Sodium Pyruvate, 1% MEM Non-essential amino-acids, 1% Glutamax, 1% Pen Strep, 0.1% Gentamycin, and 0.1% Fungizone (Invitrogen, Carlsbad CA). Frozen human PBL from patient pheresis samples were thawed and stimulated as previously described^{22, 32}.

Gamma-retroviral and Lentiviral TCR construction

MSGV1 Pmel-1 and TRP-1 TCRs—The cDNA for the native or codon optimized α and β subunits of the pmel-1 TCR (V α 1/V β 13) and the TRP-1 TCR (V α 3.2/V β 14) were linked either by a sequence encoding the foot and mouth disease picornavirus 2A ribosomal skip peptide or an internal ribosomal entry site, IRES and cloned into the MSGV1 vector.^{29, 32}

Lentiviral Pmel-1 TCR—The cDNA for the native or codon optimized α and β subunits of the pmel-1 TCR (V α 1/V β 13) were linked either by a sequence encoding the foot and mouth disease picornavirus 2A ribosomal skip peptide or an internal ribosomal entry site, IRES and cloned into a self inactivating HIV based lentiviral vector driven by a MSCV-U3 promoter.^{29, 32}

Viral Production

Platinum Eco cell lines (Cell Biolabs, San Diego, CA) were used for gamma-retroviral production while 293T cells (Invitrogen, Carlsbad, CA) were used for lentiviral production. Producer cells were plated on 10cm poly-d-lysine coated plates (BD Discovery Labware, Bellrica, MA). Transfection with appropriate DNA plasmids were done using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) as described in Figure 1.

TCR Transductions

Retronectin (Takara Bio, Otsu, Japan) and protamine sulfate (Sigma, St. Louis, MO) spinoculation protocols are described in the flow diagrams presented in Figure 2.

Adoptive Cell Transfer

Six to twelve week old C57BL/6 mice (n=5 for all groups) were injected subcutaneously with 5×10^5 B16 melanoma cells. Ten to fourteen days later, they were irradiated with 5 Gy total body irradiation (TBI) and given pmel-1 TCR or TRP-1 TCR transduced CD8⁺ or CD4⁺ T cells, respectively. T cells were generated from C57BL/6 splenocytes and administered in vivo by tail vein. Tumors were measured using digital calipers and the tumor area was calculated as the product of perpendicular diameter by investigators in a blinded manner. All experiments were performed independently at least twice with similar results and all tumor curve data is shown as mean $\pm$ standard error of the mean.

Flow Cytometry

Transduced cells were stained with fluorescently labeled antibodies for mouse anti-CD8 α , anti-CD4, anti-V β -13, anti-CD90.1 (thy1.1), anti-CD62L, anti-CD44, (BD Biosciences) and PE-labeled pmel-1 tetramer¹⁸. Human T cells were labeled with human anti-CD8 α and anti-CD4 (BD Biosciences, San Jose, CA).

Statistical Analysis

One-way ANOVA and student t-tests were used to test for significant differences. Tumor growth slopes were compared using Wilcoxon rank sum test.

RESULTS

MSGV1 vectors mediate efficient gene transfer into naïve/memory-stem cell and central memory murine T cells

To determine the ideal T cell stimulation for gene transfer, we compared anti-CD3/anti-CD28 to concavalin A (ConA)/IL-7 for 24 and 48 hours. Transduction efficiencies between the conditions were similar (39–55%; Fig. 3A). We next measured the effects of culturing T cells in different common gamma-chain cytokines and observed optimal rates of transduction when cells were cultured in IL-2 (43%) compared to IL-15 (25%) and IL-21 (9%) following initial priming with anti-CD3/anti-CD28 (Fig. 3B). We next assessed the phenotypic characteristics of the CD8⁺ T cells transduced with MSGV1 vectors. Purified populations of CD8⁺ T cells from spleens were stimulated for 24 hours with anti-CD3/anti-CD28, transduced with a MSGV1 vector encoding thy1.1 and analyzed by flow cytometry 3 and 5 days after transductions (Fig. 3C). Surprisingly, we found that 3 days following gene transfer, the majority of CD8⁺ T cells expressing thy1.1 were T_{N/SCM} (CD62L^{hi} CD44^{low}; 53%) or T_{CM} (CD62L^{hi} CD44^{hi}; 24%) cells (Fig. 3C, upper panel). After prolonging cells in culture for 5 days following gene transfer, we witnessed an increase in the percentage of thy1.1⁺ effector memory (T_{EM}, CD62L^{low} CD44^{hi}; 30%) and T_{CM} cells (65%) and a marked decrease in T_{N/SCM} cells (5%; Fig. 3C, lower panel). Thus, MSGV1 based vectors mediated efficient gene transfer into short-term cultured T_{N/SCM} and T_{CM} cells.

MSGV1 codon optimized pmel-1TCR and TRP-1 TCR vectors efficiently redirect open-repertoire CD8⁺ and CD4⁺ T cells, respectively

To develop an effective pre-clinical gene therapy model for adoptive cell transfers against established B16 melanomas, we constructed vectors using the MSGV1 backbone³³ expressing the pmel-1TCR (designated as MSGV1-pmel-1) a class I restricted receptor recognizing the melanocyte-differentiation antigen, gp100³⁴, and the TRP-1 TCR (designated as MSGV1-TRP-1), a class II restricted TCR recognizing the melanocyte-differentiation antigen, tyrosinase-related-protein-1 (Fig. 4A).³⁵ We used a sequence encoding the foot and mouth disease picornavirus 2A ribosomal skip peptide or an internal ribosomal entry site, IRES to separate the V α and V β subunits of the TCRs. The initial vectors for the pmel-1 TCR did not yield robust levels of gene expression based on flow cytometric analysis for V β 13 (MSGV1-pmel-1-2A, 17%; MSGV1-pmel-1-IRES, 13%; Fig. 4B, right upper panels) and gp100 tetramer (<1%; Fig. 4B, right lower panels). However, after codon optimization, we were able to achieve marked improvements in the surface expression of the pmel-1 TCR based on V β 13 and gp100 tetramer staining (MSGV1-pmel-1-IRES, 63% V β 13 and 39% gp100 tetramer; MSGV1-pmel-1-2A: 48% V β 13 and 32% gp100 tetramer; Fig. 4B). We next measured the efficacy of our native TRP-1 TCR constructs and attained greater than 70% TCR expression based on V β 14 staining with both the IRES and 2A vectors (Fig. 4C). Thus, both the codon optimized pmel-1 TCR and native-sequence TRP-1TCR vectors efficiently redirected TCR specificity in CD8⁺ and CD4⁺ T cells, respectively, although transduction efficiencies did appear to be slightly higher with the TRP-1 vectors.

TCR gene transduced CD8⁺ and CD4⁺ T cells mediate anti-tumor immunity

To assess the in vivo efficacy and functionality of our TCR constructs, we adoptively transferred 4×10^6 CD8⁺ T cells transduced with the MSGV1-GFP, MSGV1-pmel-1-IRES or MSGV1-pmel-1-2A vectors into sublethally irradiated (5Gy total body irradiation) mice bearing established B16 subcutaneous tumors. We observed a significant delay in tumor progression following transfer of open-repertoire CD8⁺ T cells redirected to express the pmel-1 TCR ($p < 0.05$; Fig. 5A). Both the MSGV1-pmel-1-IRES and MSGV1-pmel-1-2A vectors mediated similar degrees of anti-tumor immunity (Fig. 5A). We next measured the in vivo effects of transferring TCR redirected open-repertoire CD4⁺ T cells and observed a profound anti-tumor response with both the MSGV1-TRP-1-IRES and MSGV1-TRP-1-2A vectors following the adoptive transfer of 4×10^6 transduced cells ($p < 0.05$; Fig. 5B). Similar to experiments with the pmel-1TCR, we observed no functional differences between the TRP-1 TCR vectors encoding either the IRES or 2A linker sequences (Fig. 5B). Due to the slightly higher levels of TCR expression with the TRP-1 vectors compared to the pmel-1 vectors, we tested transferring up to 20×10^6 pmel-1 TCR transduced CD8⁺ T cells but did not observe a significant increase in anti-tumor efficacy by transferring higher number of CD8⁺ T cells (Supplementary Figure 1). Thus, CD4⁺ T cells redirected to express the TRP-1 TCR mediated robust anti-tumor immunity while pmel-1 TCR engineered CD8⁺ T cells delayed tumor growth.

Lentiviral vectors encoding the pmel-1 TCR mediate poor gene transfer in murine T cells

We next created two self-inactivating human-immunodeficient-virus (HIV) based lentiviral vectors encoding the codon optimized α and β subunits of the pmel-1 TCR linked either by a sequence encoding the foot and mouth disease picornavirus 2A ribosomal skip peptides (Fig. 6A, upper panel; designated LV-pmel-1-2A) or an internal ribosomal entry site, IRES (Fig. 6A, lower panel; designated LV-pmel-1-IRES). For comparative purposes with our gamma-retroviral vectors, we constructed lentiviral vectors driven by a MSCV-U3 promoter (Fig. 6A). We attempted to transduce open-repertoire murine CD8⁺ T cells and achieved only modest levels of gene transfer based on V β 13 specific staining with both the LV-pmel-1-2A

(11%; Fig. 6A, upper panel) and LV-pmel-1-IRES (12%; Fig. 6B, upper panel) vector. In contrast, gene transfer with the identical LV-pmel-1-2A and LV-pmel-1-IRES supernatants and similar transduction protocols led to robust V β 13 expression of 77% and 53%, respectively, in human jurkat T cells (Fig. 6B, lower panel). To confirm these surprising findings we also measured gene transfer efficiencies by staining transduced cells with a fluorescently labeled gp100₂₅₋₃₃ tetramer. Similar to the V β 13 expression pattern, gene transfer with the LV-pmel-1-IRES vector mediated poor gp100₂₅₋₃₃ tetramer staining in murine CD8⁺ T cells (3%, Fig. 6C, upper panel), but once again, robust expression was observed in human jurkat cells when using the identical viral supernatant and transduction protocol (84%, Fig. 6C, lower panel). We also tried several other strategies to improve lentiviral mediated TCR gene transfer into murine CD8⁺ T cells such as pseudo-typing the virus with an ecotropic envelope, varying the transduction protocols, and concentrating our viral supernatant, however, none of these modifications significantly improved TCR gene transfer (data not shown). Thus, lentiviral vectors mediated high levels of TCR gene transfer in human T cells, but only γ -retroviral vectors efficiently mediated TCR redirection in murine T cells.

Impaired lentiviral gene transfer specifically in murine T cells

We next wanted to investigate whether the poor lentiviral based transduction efficiencies in murine CD8⁺ T cells were a generalized phenomenon for all cell types or specific for T cells only. We used a lentiviral vector driven by MSCV-U3 promoter encoding the green fluorescent protein reporter (LV-GFP) and transduced murine 3T3 fibroblasts along with murine CD8⁺ and CD4⁺ T cells. Interestingly, the transduction efficiency in 3T3 fibroblasts was remarkably high (92%; Fig. 7A, upper left panel) compared to both murine CD8⁺ T cells (10%; Fig. 7A, upper middle panel) and murine CD4⁺ T cells (14%; Fig. 7A, upper right panel), indicating that lentiviral mediated gene transfer was impaired specifically in T cells. We next used the same LV-GFP viral supernatant and transduced human jurkat T cells along with human CD8⁺ and CD4⁺ T cells and observed excellent levels of gene transfer, 84%, 92%, and 87% respectively (Fig. 7A, lower panel). We investigated these findings quantitatively and found significantly higher GFP expression (mean fluorescence intensity; $p < 0.05$) in human compared to murine CD8⁺ and CD4⁺ T cells (Fig. 7B). Thus, the impairment in lentiviral mediated gene transfer appeared to be specific to murine T cells.

DISCUSSION

The advent of ex vivo gene transfer into lymphocytes followed by the infusion of engineered T cells in the autologous and allogeneic setting has ushered in an exciting era for gene therapy^{6, 36}. The adoptive transfer of T cells redirected to express tumor-specific TCRs represents a promising option for patients with metastatic cancer refractory to standard treatment regimens^{5, 37}. However, clinical results from gene therapy trials have thus far been disappointing^{5, 6, 19, 37, 38}.

Preclinical studies often form the basis for major advancements in biomedical research. For cancer therapeutics, developing accurate animal models closely mimicking clinical endeavors allows for the rapid testing of multiple biological parameters. We therefore attempted to generate optimized mouse models for the adoptive transfer of open-repertoire CD8⁺ and CD4⁺ T cells redirected to express tumor-specific TCRs. The stable introduction of genes into rapidly dividing cells requires an established integration of desired genetic sequences into target cells¹⁵. Both γ -retroviral and lentiviral based platforms are capable of mediating stable gene delivery into mammalian cells, but marked heterogeneity exists in regard to optimal vector backbones depending on differences in recipient species and cell types^{14, 16, 39, 40}. Lentiviruses are quickly becoming the standard for mediating gene transfer in human lymphocytes^{20, 28, 29, 32, 41}. These vectors mediate high transduction

efficiencies, stable integration, and possess the ability to transfer genes into resting lymphocytes^{21, 41}. While much work has been conducted on human lymphocytes, the ability of lentiviral based vectors to mediate gene transfer in murine T cells has not been thoroughly explored. In contrast, γ -retroviral vectors mediate efficient delivery of genes into murine T cells but the requirement for a strong stimulus and active cell division has led to the general belief that transducing less differentiated T cells may not be possible.

We therefore developed both γ -retroviral and lentiviral vectors to try to redirect TCR specificity of murine T cells to recognize tumor antigens expressed by B16 melanomas. During optimization, we observed that 24 hour stimulation with anti-CD3 and anti-CD28 in the presence of IL-2 provided sufficient priming of T cells for efficient gene delivery. Under these culture conditions, we surprisingly found that MSGV1 based vectors effectively transduced both naïve/memory-stem cell and central memory T cells. Most likely, the short 24 hour stimulation prevented early differentiation of naïve/memory-stem cell and central memory T cells into effectors, yet still pushed T cells into active cell division, making them permissible for retroviral integration.

We previously found that the pmel-1 TCR on a PMX backbone led to modest levels of anti-tumor immunity and therefore looked to improve upon earlier results¹⁸. We optimized gamma-retroviral transduction conditions and constructed vectors encoding for the pmel-1 and TRP-1 TCR on a MSGV1 backbone in order to redirect both CD8⁺ and CD4⁺ T cells to recognize the melanocyte differentiation antigens gp100 (class I restricted) and tyrosinase-related-protein-1 (class II restricted), respectively. We found it necessary to codon optimize the sequences for the alpha and beta subunits of the pmel-1 TCR in order to achieve robust gene expression. Interestingly, we did not need to codon optimize the TRP-1 TCR sequences and obtained the highest levels of TCR expression with these vectors. The biological reasons for these differences remain unclear, although previous studies have described the existence of 'strong' and 'weak' TCRs in regard to surface expression^{42, 43}.

For functional examination of the TCR vectors, we adoptively transferred CD8⁺ T cells engineered to express the pmel-1 TCR and CD4⁺ T cells engineered to express the TRP-1 TCR into mice bearing established subcutaneous B16 tumors. We observed a delay in tumor growth with pmel-1 TCR transduced CD8⁺ T cells and striking anti-tumor immunity with TRP-1 TCR engineered CD4⁺ T cells. The differences in treatment could not be explained by the increased levels of TRP-1 TCR expression because transferring higher numbers of pmel-1 TCR engineered CD8⁺ T cells (up to 20 X 10⁶) did not appear to induce similar degrees of anti-tumor immunity observed after transferring TRP-1 TCR engineered CD4⁺ T cells. We and others have described the importance of CD4⁺ T cells as potent and direct mediators of tumor destruction, however, it remains unclear whether the differences in tumor treatments were due to the inherent anti-tumor abilities of CD8⁺ versus CD4⁺ T cells or intrinsic differences in the TCRs^{35, 44, 45}. Nevertheless, these are much needed models to perform mechanistic studies to improve the efficacy of current TCR gene therapy clinical trials⁴⁶ and this study represents the first description of adoptively transferring genetically engineering CD4⁺ T cells expressing the TRP-1 TCR.

A recent report by Bendle et al. described toxicities after adoptively transferring TCR engineered T cells in vivo⁴⁷. However, we did not see any clear signs of toxicity with the pmel-1 TCR due to the continued growth of tumors precluding any long-term studies. We did note evidence of long term weight loss in some mice following treatment with TRP-1 TCR transduced CD4⁺ T cells, but need to further investigate the nature of the weight loss.

In this study, we also created HIV-based self-inactivating lentiviral vectors encoding the pmel-1 TCR and unexpectedly found low levels of TCR gene expression in murine T cells.

In contrast, human T cells engineered with the lentiviral pmel-1TCR efficiently expressed high levels of the murine receptor. To test if this defect was specific for T cells, we simultaneously transduced murine fibroblasts and murine CD4⁺/CD8⁺ T cells with a LV-GFP construct and demonstrated high levels of gene transfer in only the fibroblasts, indicating that murine T cells possessed inherent characteristics preventing lentiviral infection. We tried several different transduction protocols in addition to concentrating our virus but were unable to obtain gene transfer efficiencies comparable to γ -retroviral vectors. The lentiviral vectors we constructed were driven by a MSCV-U3 promoter to allow for direct comparisons with our γ -retroviral vectors. In the future, one possible strategy to boost lentiviral transductions in murine T cells might be to use different promoters⁴⁸. However, based on our ability to efficiently mediate gene transfer into T_{N/SCM} and T_{CM} cells with γ -retroviral vectors, the advantages of optimizing lentiviral based gene transfer into minimally stimulated murine T cells may not be required.

In summary, HIV-based lentiviral vectors may not represent the ideal system to mediate robust TCR gene transfer in murine T cells. The most likely explanation for this phenomenon can be drawn from studies by Baumann et al.⁴⁹ and Tsurutani et al.⁵⁰ that describe reduced levels of HIV infectivity in murine T cells due to lower efficiencies of reverse transcription and nuclear translocation. Understanding and developing techniques to overcome these limitations would be valuable for scientists across multiple disciplines. For performing pre-clinical adoptive immunotherapy studies, utilizing lentiviral vectors is challenging due to the large number of cells needing to undergo transduction prior to cell transfer. One strategy to overcome the limitations of reduced levels of TCR gene transfer is to perform a cell sort prior to adoptive transfer experiments, but this would dramatically increase the cost of performing in vivo experiments. In conclusion, for practical reasons, we recommend using gamma-retroviral vectors for future pre-clinical adoptive immunotherapy studies requiring the genetic engineering of murine T cells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research.

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FIGURE 1.
Flow diagram for gamma-retroviral and lentiviral production.

GENE TRANSDUCTION PROTOCOLS

FIGURE 2.
Flow diagram for gene transduction protocols

FIGURE 3.

Optimization of gene transfer into T cells with gamma-retroviral vectors. A, Bulk C57BL/6 splenocytes were stimulated with either anti-CD3 and anti-CD28 or con A and IL-7 for 24 or 48 hours and transduced with the MSGV1-GFP vector. B, Congenically marked (CD45.1) CD8⁺ T cells isolated from C57/BL-6 spleens were stimulated with anti-CD3 and anti-CD28 and cultured in IL-2, IL-15, or IL-21 and transduced with the MSGV1-GFP vector. Transduction efficiencies for A and B were measured by flow cytometry 3 days following gene transfer (grey = non-transduced control). C, CD8⁺ T cells were stimulated with anti-CD3/anti-CD28 for 24 hrs, cultured in IL-2, transduced with a MSGV1-thy1.1 vector and examined by flow cytometry 3 and 5 days after transduction for CD62L and CD44 to define T_N/SCM (CD62L^{Hi} CD44^{Low}) and T_{CM} (CD62L^{Hi} CD44^{Hi}) populations. All results in A, B, and C are representative of at least 3 independent experiments.

FIGURE 4.

Redirection of open-repertoire CD8⁺ and CD4⁺ T cells with MSGV1 vectors encoding the codon optimized pmel-1 and native TRP-1 TCRs. A, The α and β subunits of the pmel-1 TCR (Vα1/Vβ13) and the TRP-1 TCR (Vα3.2/Vβ14) were linked either by a sequence encoding the foot and mouth disease picornavirus 2A ribosomal skip peptide (upper panels) or an internal ribosomal entry site, IRES (lower panels) and cloned into the MSGV1 vector backbone (SD, splice done; SA, splice acceptor; LTR, long terminal repeat; Ψ, packaging sequence). B, C57BL/6 splenocytes were stimulated with anti-CD3/anti-CD28 for 24 hrs, cultured in IL-2, and transduced with the MSGV-1 vectors encoding for either the native or codon optimized pmel-1 TCR sequences containing IRES or 2A. Transduction efficiencies in CD8⁺ T cells were measured by flow cytometry for Vβ13 (upper panel) or gp100₂₅₋₃₃ tetramer expression (lower panel). C, C57/BL-6 splenocytes were stimulated with anti-CD3/anti-CD28 for 24 hrs, cultured in IL-2, and transduced with the MSGV1-TRP-1-IRES and MSGV1-TRP-1-2A vectors. TCR transfer efficiencies in CD4⁺ T cells were measured by flow cytometry for Vβ14. Mock represents cells taken through the transduction protocol with complete media instead of viral supernatant. All results A, B, and C, are representative at least 3 independent experiments.

FIGURE 5.

Anti-tumor immunity of CD8⁺ T cells engineered to express the pmel-1 TCR and CD4⁺ T cells transduced with the TRP-1 TCR. A, Tumor treatment following the adoptive transfer of 4×10^6 open repertoire CD8⁺ T cells transduced with the MSGV1-GFP, MSGV1-pmel-1-IRES, or MSGV1-pmel-1-2A vectors into sublethally-irradiated (5 Gy) mice bearing B16 tumors ($n=5$) established for 10 days [* , ** = $p < 0.05$ compared to no treatment (NT)] B, Anti-tumor immunity following the adoptive transfer of 4×10^6 open repertoire CD4⁺ T cells transduced with the MSGV1-GFP, MSGV1-TRP-1-IRES, or MSGV1-TRP-1-2A vectors into sublethally-irradiated (5Gy) mice bearing B16 tumors ($n=5$) established for 10 days [* , ** = $p < 0.05$ compared to no treatment (NT)]. All results for (A) and (B) are representative of at least two separate experiments.

FIGURE 6.

Gene transfer with HIV based self-inactivated lentiviral vectors encoding the pmel-1 TCR. A, The HIV based lentiviral vector encoding the pmel-1 TCR linked by either a 2A linker (upper panel) or an internal ribosomal entry site, IRES (lower panel) (LTR, long terminal repeat; Ψ, packaging sequence; cPPT, central polypurine tract; RRE, rev response element; MSCV-U3, murine-stem-cell-virus-based promoter). B, Open-repertoire murine CD8⁺ T cells from spleens were stimulated with anti-CD3 and anti-CD28 and transduced with the LV-Pmel-2A and LV-Pmel-IRES vectors. Human jurkat T cells were transduced with the same viral supernatant. Transduction efficiencies were analyzed by flow cytometry for Vβ-13 expression. C, Transduced murine CD8⁺ T cells and human jurkat T cells from B, were analyzed by flow cytometry for gp100₂₅₋₃₃ tetramer expression. Mock represent cells taken through the transduction protocol with complete media instead of viral supernatant. All results for A, B, and C, are representative of at least 2 independent experiments.

FIGURE 7.

Comparative transductions between murine and human cells with lentiviral vectors. A, Murine fibroblasts, CD8⁺ and CD4⁺ T cells, along with human jurkat, CD8⁺ and CD4⁺ T cells were transduced with a HIV based lentivirus encoding the green fluorescent protein (GFP). Murine and human T cells were stimulated similarly with anti-CD3 and anti-CD28 and cultured in IL-2. Transduction efficiencies in cells were analyzed by flow cytometry for GFP expression. Results are representative of at least 2 independent experiments. B, The mean fluorescence intensity (MFI) was calculated and quantified for GFP transduced murine and human CD8⁺ and CD4⁺ T cells (*, ** p < 0.05; n=3). All results for A and B are representative of at least 2 independent experiments.