Published in final edited form as:

Cancer Gene Ther. 2015 July ; 22(7): 360–367. doi:10.1038/cgt.2015.28.

IL-12-conditioning improves retrovirally-mediated transduction efficiency of CD8+ T cells

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Abstract

The ability to genetically modify T cells is a critical component to many immunotherapeutic strategies and research studies. However, the success of these approaches is often limited by transduction efficiency. Since retroviral vectors require cell division for integration, transduction efficiency is dependent on the appropriate activation and culture conditions for T cells. Naïve $CD8^+$ T cells which are quiescent must be first activated to induce cell division to allow genetic modification. To optimize this process, we activated mouse T cells with a panel of different cytokines, including IL-2, IL-4, IL-6, IL-7, IL-12, IL-15 and IL-23, known to act on T cells. After activation, cytokines were removed, and activated T cells were retrovirally transduced. We found that IL-12 pre-conditioning of mouse T cells greatly enhanced transduction efficiency while preserving function and expansion potential. We also observed a similar transduction enhancing effect of IL-12 pre-conditioning on human T cells. These findings provide a simple method to improve the transduction efficiencies of $CD8^+$ T cells.

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Conflict of Interest:
We have no conflicts of interest to declare.

Introduction

The genetic modification of T cells is a critical methodological step in both medicine and science[1–4]. The adoptive transfer of T cells can mediate potent anti-tumor and anti-viral immunity in patients[3–14]. Such therapy may depend on the transfer of genetic information including T-cell receptors (TCRs), chimeric antigen receptors (CARs), or other effector molecules[3–14]. The genetic modification of T cells is also an important tool for studying the function of genes in basic science and translational research. These approaches are all dependent on achieving efficient transduction and the extended culture of T cells.

The transduction efficiency of commonly used retroviral vectors, including those based on the Moloney murine leukiema virus (MoMLV), is dependent on cell division[15, 16]. In the case of T cells, which are normally quiescent and non-dividing, this means appropriate activation and culture conditions are essential for not only allowing gene transduction, but also expanding T cells to adequate numbers for downstream applications. Most commonly, mouse T cells are activated by engaging the TCR (signal 1) and CD28 costimulatory molecule (signal 2) with antibodies against CD3 and CD28, respectively, followed by culture with IL-2[17]. This methodology allows for efficient activation of T cells, cell division, and ultimately, the expansion of large numbers of T cells. With mouse T cells, there is a bias towards expansion of $CD8^+$ T cells[18]. While IL-2 is traditionally used to culture T cells, many other cytokines play an important role in impacting T cell proliferation, survival, and function. We and others have found that conditioning T cells with IL-12 during activation greatly improves $CD8^+$ T cell persistence and anti-tumor efficacy[19–22]. IL-23 is in the same family as IL-12, and also acts directly on T cells and has a notable role in supporting Th17 cells[23–25]. Another cytokine, IL-6, can also directly act on T cells, and has shown to act as a costimulatory molecule and impact T cell survival[26–28]. Finally, there has been extensive research demonstrating that members of the IL-2Rγ-chain family including IL-4, IL-7 and IL-15, can play an important roles in multiple aspects of T cell function including survival and proliferation[29–31].

We hypothesized that distinct cytokines would not only differentially impact the survival and functional outcome of T cells but also regulate transduction efficiency. To determine if the provision of specific cytokines during T cell activation could regulate or improve transduction efficiency, we activated mouse T cells with anti-CD3 mAb and anti-CD28 mAb for 48 hours using the following cytokines: IL-2, IL-4, IL-6, IL-7, IL-12, IL-15, and IL-23. After washing out the cytokine, T cells were retrovirally transduced and cultured in IL-2. After ~1 week, we assayed the T cells for transduction efficiency. T cells pre-conditioned with IL-12 exhibited greatly improved transduction efficiency. This was associated with maintenance of function as determined by the ability of TCR-modified T cells to recognize cognate antigen. Furthermore, IL-12-conditoned T cells were able to expand in a similar manner to control cells without conditioning. We also found that IL-12 conditioning was associated with enhanced Bcl-3 mRNA expression, suggesting a mechanism for the improvement in transduction efficiency. Our findings demonstrate that the addition of IL-12 to T cell cultures provides a simple way to greatly improve retroviral-mediated genetic modification.

Materials and methods

Generation of retroviral supernatant and retroviral vectors

For mouse T cells, we used retroviral vectors encoded by the following plasmids: (MSCV) Tyr-TCR/s39TK-GFP vector (kindly provided by A. Ribas)[32], MSCV-GFP and MSCV-Tbet/GFP (were kindly provided by L. Gapin with the permission of L. Glimcher)[33], and MSGV-1D3-28Z.1-3[34]. To generate retroviral supernatant, PLAT-E cells were transfected using Lipofectamine 2000 (Invitrogen, Grand Island, NY). Media was changed 6 hours after addition of Lipofectamine 2000, and viral supernatant was harvested at 24–72 hours post-transfection. For human T cells, we used a PG13 packaging cell clone (22M) which was transfected with the TIL1383I TCR/CD34t plasmid which encodes the TIL1383I TCR and a truncated CD34 molecule[35]. The 22M packaging clone was kindly provided by M. Nishimura (Loyola University, Chicago, IL).

T cell culture, transduction, and purification

Unless specified otherwise, C57BL/6 (B6) splenocytes were activated with anti-CD3 mAb (145-2C11 clone, plate-bound, 1ug/ml) and anti-CD28 mAb (37.51 clone, soluble, 2ug/ml) in RPMI media for 48 hours with or without additional cytokines including: human (h) IL-2 (200ng/ml), mouse (m) IL-4 (100ng/ml), hIL-6 (25ng/ml), mIL-7 (100ng/ml), mIL-12 (10ng/ml), mIL-15 (200ng/ml), and mIL-23 (10ng/ml). After 48 hours, cells were washed, mixed with 50% viral supernatant, 50% fresh RPMI media supplemented with 200ng/ml hIL-2 and spinoculated on Retro-Nectin (Takara, Mountain View, CA) coated non-tissue culture plates at 3000 g at 32°C for 2 hours. After 24 hours, the transduction step was repeated. The next day, cells were washed, and maintained in culture between 8×10^5 and 1×10^6 cells/ml with hIL-2 (200ng/ml) in 24-well plates in 1.5ml. Cells were assayed for transduction efficiency and function by flow cytometry 48–72 hours after the second transduction. In Supplemental figure 2, T cells were purified prior to activation and transduction. $CD3^+$ T cells were enriched from splenocytes using a T cell enrichment column (MTCC-25, R&D Systems, Minneapolis, MN). In a second step, $CD8^+$ T cells were then further purified by negative selection using magnetic beads (MAGM203, R&D Systems). For transduction of human T cells, we used a modification of a previously described protocol[35]. Briefly, we obtained de-identified PBMCs from Research Blood Components (Boston, MA). Human PBMCs were cultured with hIL-2 (300 IU/ml) and hIL-15 (100ng/ml) and stimulated with anti-CD3 mAb (OKT3) for two days with or without IL-12 (10ng/ml). On day 3, cells were transduced on retronectin-coated plates and underwent spinoculation (2000g for 2 hours at 37°C), and were then maintained with hIL-2 (300IU/ml) and hIL-15 (100ng/ml) until analysis.

Flow cytometry

Cells were analyzed by flow cytometry using standard procedures as previously described[19]. Briefly, cells were washed in staining buffer (PBS, 2% bovine growth serum and 0.01% sodium azide) and stained with fluorescently labelled antibodies. The fluorescently conjugated antibodies used in this study included: anti-mouse (m) CD3 mAb (145-2C11), anti-mCD4 mAb (RM4-5), anti-mCD8 mAb (53-6.7), anti-mCD25 mAb (PC61), anti-mIFNγ mAb (XMG1.2), anti-hVβ12 (VER2.32.1), anti-human (h) CD4 mAb (OKT4), anti-

hCD8 mAb (RPA-T8), and anti-hCD34 mAb (581). For staining mouse T cells transduced with the Tyr-TCR/s39TK-GFP vector, we also used HLA-A-0201-H-2K^b chimera/YMDGTMSQV tetramer derived from the human Tyrosinase$_{368-376}$ (hTyr) peptide. This tetramer was conjugated to ALX647 and kindly provided by the NIH tetramer facility. To determine the transduction efficiency of CAR-mediated retroviral modification, cells were stained with Biotin-Protein L reagent (M00097, GenScript, Piscataway, NJ). The lyophilized form of this product was reconstituted in deionized water to a final concentration of 1 mg/ml and stored at −80°C. In combination with Biotin-Protein L, streptavidin-APC was used to detect CAR expression on the cell surface. For intracellular cytokine expression, we used the BD Cytofix/Cytoperm Kit (BD Bioscience, San Jose, CA). Samples were acquired on BD Accuri and data was analyzed using Flowjo software (TreeStar, Ashland OR) and CFLOW software (BD Bioscience).

T cell functional assays

To assay the ability of T cells to respond functionally to antigen, we co-cultured mouse TCR-modified T cells (10^5) with T2-A2 cells (10^5) which express HLA-A2. Cultures were set up with or without 1ug/ml hTyr peptide (YMDGTMSQV, American Peptide Company) in a flat-bottom 96-well plate. After incubation for 6 hours at 37°C, cells were stained for intracellular cytokines.

RNA isolation and Real-time PCR

Total cellular RNA was isolated from CD8$^+$ T cells cultured with and without IL-12 using Trizol reagent (Life technologies, Grand Island, NY). cDNA was generated from 1μg of total RNA using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). The resulting cDNA was amplified by qRT-PCR using primer pairs for the Bcl-3 (NM_033601 forward 5'-CCGGAGGCCCTTTACTACC-3'; reverse 5'-GAGTAGGCAGGTTCAGCAGC-3'), CAT1 (NM_007513 forward 5'-ACAGCAGAGTCCACGGTAGC-3'; reverse 5'-AGCTAGCAGGTGTGAGAGGC-3'), 18S rRNA (K01364 forward 5'-CCAGAGCGAAAGCATTTGCCAAGA-3'; reverse 5'-TCGGCATCGTTTATGGCTGGAACT-3'), and beta-actin (NM_007393 forward 5'-ACGTAGCCATCCAGGCTGGTG-3'; reverse 5'-TGGCGTGAGGGAGAGCAT-3') using Sso advance SYBR green on CFX96 touch real-time PCR detection system (Bio-Rad, Hercules, CA). The levels of Bcl-3 and CAT1 in each sample were normalized to beta-actin and 18S rRNA expression. The final relative expression of mRNA species was calculated using the comparative Ct method.

Statistical Analysis

Data were graphically displayed to identify the need for transformation prior to analysis. Differences between % positive cells (GFP in Figure 2; CD34 in Figure 5) with and without IL-12 were compared using paired t-tests with a two-sided alpha of 0.05. To evaluate fold-change relative to control (Figures 4D and 4F), a GEE estimation approach was used to account for correlation due to batch effects. An intercept only linear regression with log fold-change as the outcome was estimated and the p-value for the intercept reported.

Results

Pre-conditioning with IL-12 during T cell activation enhances transduction efficiency

To determine if pre-conditioning with cytokines could improve transduction efficiency without decreasing functional ability, B6 splenocytes were activated for 48 hours with one of the following cytokines: hIL-2, mIL-4, hIL-6, mIL-7, mIL-12, mIL-15, and mIL-23 as shown in Figure 1A. Cells were then washed and re-cultured from days 2 to 7 with IL-2. On days 2 and 3, cells were retrovirally transduced with the Tyr-TCR/s39TK-GFP MoMLV-based vector encoding both a tyrosinase-reactive HLA-A2-restricted human T-cell receptor (TIL1383I) and GFP (Figure 1B). These genes allowed the direct assessment of the ability of T cells to function in an antigen-specific manner and a simple method for the identification of transduced T cells based on GFP fluorescence. Upon evaluation of the 7 different cytokines, we found that pre-conditioning with IL-12 was able to improve transduction efficiency by roughly 50% as indicated by retrovirally-encoded GFP expression (Figure 1C). Importantly, IL-12-pre-conditioned cells maintained their ability to respond functionally to relevant antigen as indicated by staining for intracellular IFNγ expression after stimulation for 6 hours with relevant antigen (hTyr peptide in the context of HLA-A2) (Figure 1D).

Pre-conditioning with IL-12 enhances the transduction efficiency of $CD8^+$ T cells

We observed enhanced transduction efficiency with IL-12-conditioning reproducibly in over 10 experiments (Figure 2A). While the enhanced transduction efficiency was most evident in the $CD8^+$ T cell compartment, we also observed enhanced transduction efficiency in the $CD4^+$ T cell compartment (Figure 2B, C, D). Whereas GFP is one measure of transduction efficiency, we also assayed the expression of the retrovirally-encoded TCR using antibodies against hVβ12 and an HLA-A2 tyrosinase tetramer. Using both staining methodologies, we observed enhanced transduction efficiency (Figure 2E, F). We also observed antigen-induced intracellular IFNγ expression selectively in the GFP^+ subset of the transduced $CD8^+$ T cells and this correlated with the IL-12-mediated enhanced transduction efficiency (Figure 2G). As a control, IL-12-conditioned T cells cultured without antigen did not produce IFNγ.

Enhanced transduction with IL-12-preconditioning is not dependent on culture conditions or a specific retroviral vector

To ensure that our cell culture conditions were not uniquely promoting enhanced IL-12-mediated transduction, we examined several variables. Modifying the method of T cell activation by using plate-bound anti-CD28 mAb rather than using soluble antibody did not impact transduction efficiency nor did using bead-bound instead of plate-bound CD3/CD28 (Figure S1A and data not shown). Furthermore, reducing the number of T cells during the transduction step improved efficiency, but this was secondary to the impact of IL-12 (Figure S1B). This latter finding may reflect the ability of T cells to divide more or an increased concentration of viral vectors. The improved transduction efficiency with IL-12 pre-conditioning was also apparent with a 10-fold lower concentration of IL-2 (Figure S1C), and thus not likely to be a consequence of altered IL-2 responsiveness after the transduction step. Importantly, we also found that when we started the transduction protocol with enriched $CD3^+$ T cells or purified $CD8^+$ T cells, there was enhancement of transduction efficiency

with IL-12 pre-conditioning (Figure S2). Thus as previously shown[22], our data are consistent with IL-12 acting directly on the T cells and not acting through accessory cells.

To verify that the enhanced transduction efficiency was not vector specific, we transduced T cells with a total of four different retroviral vectors. Three of the vectors (Tyr-TCR/s39TK-GFP, MSCV-GFP, MSCV-Tbet/GFP) are derived from the MSCV retroviral backbone, and one of them (MSGV-1D3-28Z.1-3) is derived from the MSGV viral backbone. In all cases, pre-conditioning of T cells with IL-12 led to markedly improved transduction efficiency (Figure 3, and S3). This was measured by staining cells for GFP expression in the MSCV-derived vectors (Figure 3), or in the case of the MSGV-1D3-28Z.1-3 vector, which expresses a CD19-reactive chimeric antigen receptor (CAR); cells were stained with the biotin-protein L reagent (Figure S3). It is notable that we were able to detect CAR-modified T cells using a very low dilution of this reagent[36].

Pre-conditioning with IL-12 directly improves transduction efficiency and does not impair cellular expansion

While our data suggest that IL-12 improved transduction efficiency, it was possible that IL-12 may enhance retroviral-mediated gene expression after transduction. To address this, we cultured T cells with IL-12 either before (early) or after (late) transduction (Figure 4A). Cells cultured with IL-12 added after transduction did not show a significant increase in retroviral-mediated gene expression, thus, supporting our hypothesis that IL-12-condtioning was directly leading to improved transduction efficiency. As a control in this experiment, only late IL-12 conditioning increased expression of IL-2Rα (CD25) as expected[37].

An important methodological component of our study was whether IL-12 conditioning impacts the ability of T cells to expand or alter the CD8 to CD4 ratio. We found that T cells conditioned with IL-12 were able to expand logarithmically (Figure 4B). Furthermore, IL-12 conditioning did not alter the CD8 to CD4 ratio (Figure 4C). Together, these results demonstrate that this methodological change does not detrimentally impact the ability to efficiency generate retrovirally-transduced $CD8^+$ T cells.

IL-12-conditioning enhances Bcl-3 mRNA expression but not the CAT-1 ecotropic receptor

To understand the mechanism by which IL-12 conditioning improves transduction efficiency, we tested whether Bcl-3, a known IL-12 target[21, 38], is upregulated in our T cell cultures after cytokine conditioning. Using real time PCR to assess RNA levels, we found that Bcl-3 was significantly higher in cells conditioned with IL-12 (Figure 4D). We also assessed whether IL-12 conditioned T cells had improved viability or proliferation by staining with 7-AAD and BrdU, and also assessing FS/SC by flow cytometry. We did not observe significant differences in viability or proliferation as a result of IL-12 conditioning (Figure 4E and data not shown), suggesting that Bcl-3 may be inducing more subtle changes than apparent by direct functional assay. It is also possible that our assays were not timed optimally. Given that other investigators have linked IL-12 with improved survival[21, 22, 38], our data are consistent with the possibility that IL-12-mediated improved survival could contribute to the enhanced transduction efficiency.

Another possibility of how IL-12 could improve transduction efficiency is modulation of expression of the ecotropic receptor (CAT-1/Slc7a1) necessary for binding and internalization of the retroviral vectors[39–41]. We assessed CAT-1 expression with or without IL-12 conditioning. We did not observe an IL-12-mediated effect on CAT-1 mRNA levels (Figure 4F) suggesting that modulation of the ecotropic receptor at the RNA level is not likely to account for the improved transduction efficiency.

Human CD8$^+$ T cells pre-conditioned with IL-12 have improved transduction efficiency

While the ability to efficiently transduce mouse T cells is useful, from a clinical perspective, transduction of human T cells is critical. Therefore, we evaluated whether we could improve the transduction efficiency of human T cells using a clinically relevant protocol. Human PBMCs were stimulated with soluble anti-CD3 mAb (OKT3 clone) with or without IL-12 conditioning. After removal of IL-12, cells were washed, and transduced with a vector encoding both the TIL1383I TCR and a truncated CD34 molecule. As shown in Figure 5A, pre-conditioning with IL-12 greatly enhanced the transduction efficiency of human CD8$^+$ T cells as indicated by expression of CD34. This was apparent from multiple experiments (Figure 5B). Importantly, IL-12 conditioning did not impair the ability of human T cells to expand effectively (data not shown).

Discussion

In this study, we have shown that pre-conditioning of murine and human CD8$^+$ T cells with IL-12 greatly improved transduction efficiency. Importantly, the improved transduction efficiency was associated with maintenance in both function and expansion potential. The improved transduction efficiency was not dependent on specific culture conditions as we observed improved efficiency with different culture methodology as well as with different retroviral vectors. Together, our results demonstrate that the brief addition of IL-12 to T cells during activation provides a reliable method to improve transduction efficiency.

To explain the mechanism of enhanced IL-12-mediated transduction efficiency, we assayed a number of parameters. As a result of IL-12 conditioning, we observed significant elevation in Bcl-3 mRNA expression by quantitative PCR. We did not observe IL-12-dependent improved viability or proliferation in our cultures. However, as IL-12 has been reported by several investigators to improve both Bcl-3 expression and survival in T cells[21, 38], it is likely that our functional assays assessing survival may have not been sensitive enough or timed correctly. Overall, our assays are consistent with the possibility that IL-12-mediated improved survival contributes to the enhanced transduction efficiency, but other pathways may also be relevant.

An alternate pathway that could explain the improved transduction efficiency of T cells is by enhancing the expression of the retroviral receptor necessary for binding and internalization of the retroviral vector. For the mouse ecotropic vector, this receptor is CAT-1[39–41]. We assayed the expression of CAT-1 mRNA by quantitative PCR. However, the levels of CAT-1 were not changed by IL-12 conditioning. Consistent with this, we also failed to see significant changes in CAT-1 by analysis of publically available gene chip data[42]. Thus, if CAT-1 is relevant, it is not due to regulation of the level of RNA expression.

Although we did not find direct evidence of a mechanism of improved transduction efficiency, IL-12 conditioning impacts the expression of a very large number of genes, and there may be other pathways contributing to the improved transduction efficiency. For example, IL-12 conditioned T cells have greatly elevated levels of granzyme B and other molecules important for effector function[19, 43, 44]. These may be relevant for the efficiency of retroviral transduction. These possibilities could be addressed by utilization of T cells from gene knockout mice.

Altogether, our results demonstrate a simple method for improving the transduction efficiency of mouse and human $CD8^+$ T cells. From a practical standpoint, our finding will facilitate the utilization of retroviral vectors to genetically modify $CD8^+$ T cells. If future studies can more fully elucidate the relevant mechanisms by which IL-12-conditioning improves transduction efficiency, we may be able to use this mechanistic information to develop improved methods for the transduction of T cells or other cell populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Dan Neitzke for critical review of this manuscript. We thank Gina Scurti (Loyola University) for expert technical advice in culturing human T cells. Grant funding for this project was provided by P01CA54778-01 from the National Institutes of Health and the National Cancer Institute. This work was also supported in part by the Cell Evaluation & Therapy Shared Resource, Hollings Cancer Center, Medical University of South Carolina (P30CA138313). We also acknowledge the NIH Tetramer Core Facility (contract HHSN272201300006C) for provision of the tetramer used in this study.

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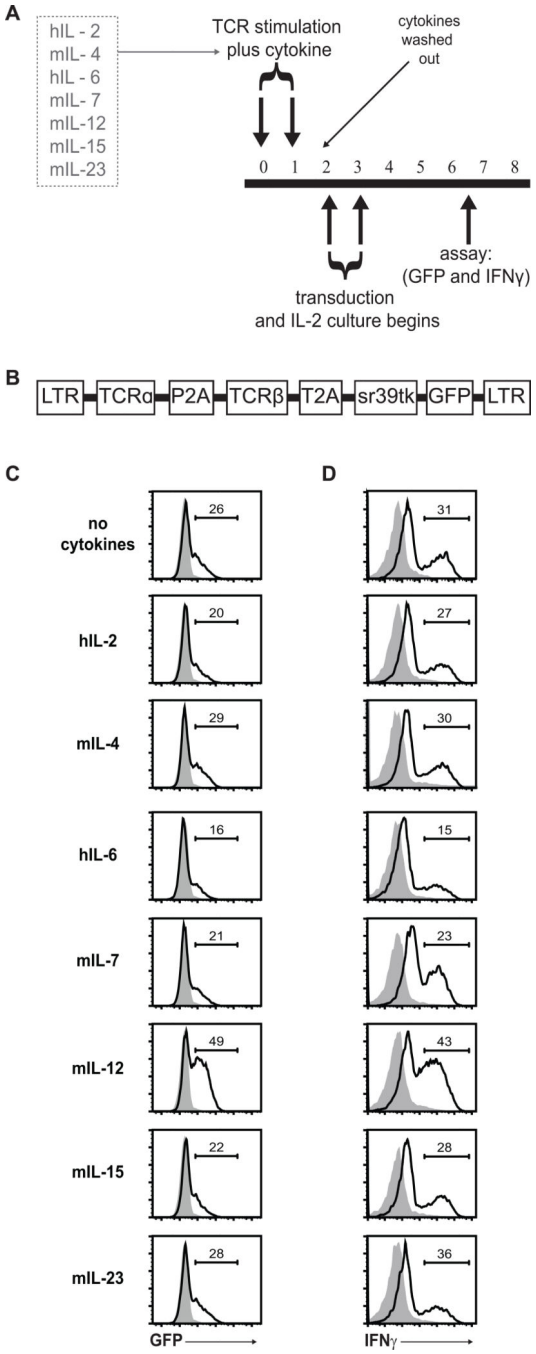

Figure 1. Identification of IL-12 as a key cytokine for enhancing transduction efficiency when added during T cell activation

(A) To obtain retrovirally modified T cells, B6 splenocytes were activated with anti-CD3 mAb and anti-CD28 mAb for 48 hours with or without additional cytokines. After 48 hours, cells were washed and recultured in IL-2. On day 6 and 7, cells were assayed phenotypically and functionally. (B) Shows a diagram of MSCV-derived Tyr-TCR/s39TK-GFP vector containing the tyrosinase-reactive TCR αβ genes. (C) On day 6 of culture, retrovirally modified T cells were assayed by flow cytometry for GFP expression. The black line shows transduced T cells, and the shaded histogram shows control (non-transduced) T cells. The

number shows the percentage of GFP^+ cells. (D) On day 7 of culture, retrovirally modified T cells were assayed for functional ability by co-culture with T2-A2 antigen presenting cells with or without hTyr peptide. After 6 hours, cells were stained for intracellular IFNγ expression. All data are representative of two independent experiments.

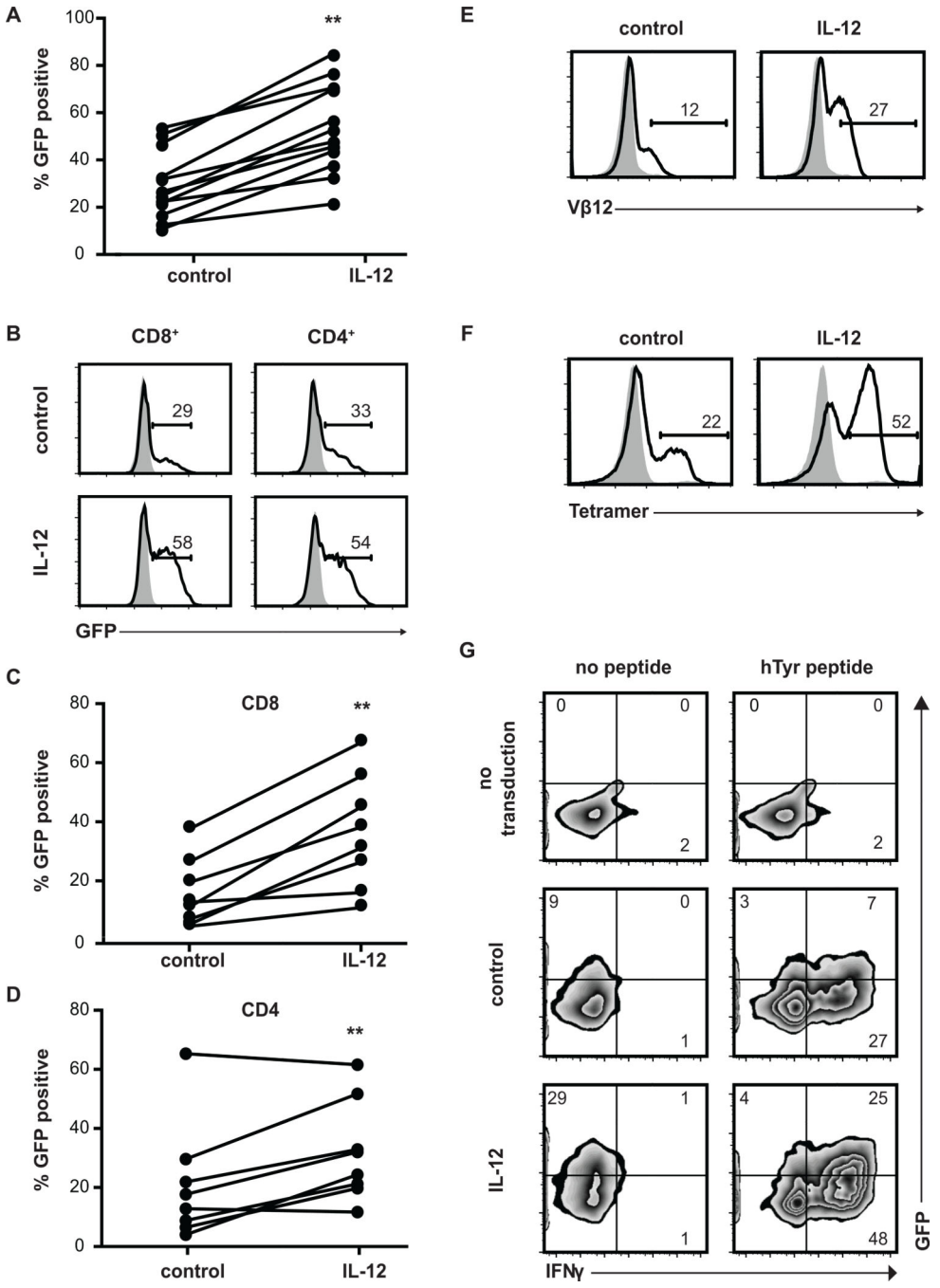

Figure 2. IL-12 conditioning during activation enhances the transduction efficiency of mouse CD8$^+$ and CD4$^+$ T cells

(A) Murine T cells were activated with anti-CD3 mAb (plate-bound) and anti-CD28 mAb (soluble) for 48 hours with or without IL-12 conditioning. Cells were then washed, transduced with the Tyr-TCR/s39TK-GFP vector, and recultured in IL-2. On day 7, the frequency of transduced T cells was determined by evaluation of GFP expression by flow cytometry. Results from eleven independent experiments are shown. The line connects points from the same experiment. (B) Shows representative FACS staining from 'A', gated on CD8$^+$ (left) or CD4$^+$ (right) positive T cells. The black line shows transduced T cells, and

the shaded histogram shows control untransduced T cells. The number shows the percentage of GFP^+ cells. (C) As in 'A', except analysis of transduction efficiency among $CD8^+$ T cells gated from eight independent experiments. (D) As in 'A', except analysis of transduction efficiency among $CD4^+$ T cells gated from eight independent experiments. (E) As in 'B', except cells were stained for hVβ12 expression. (F) As in 'B', except cells were stained for expression of the retrovirally-encoded TCR using a tetramer. (G) Transduced T cells (or control T cells) were stimulated with or without hTyr peptide for 6 hours with T2-A2 cells, and assayed by flow cytometry for GFP and intracellular IFNγ expression. For A, C, and D, (**) indicates a significant difference ($p \leq 0.01$) between conditions with or without IL-12 conditioning based on paired t-tests.

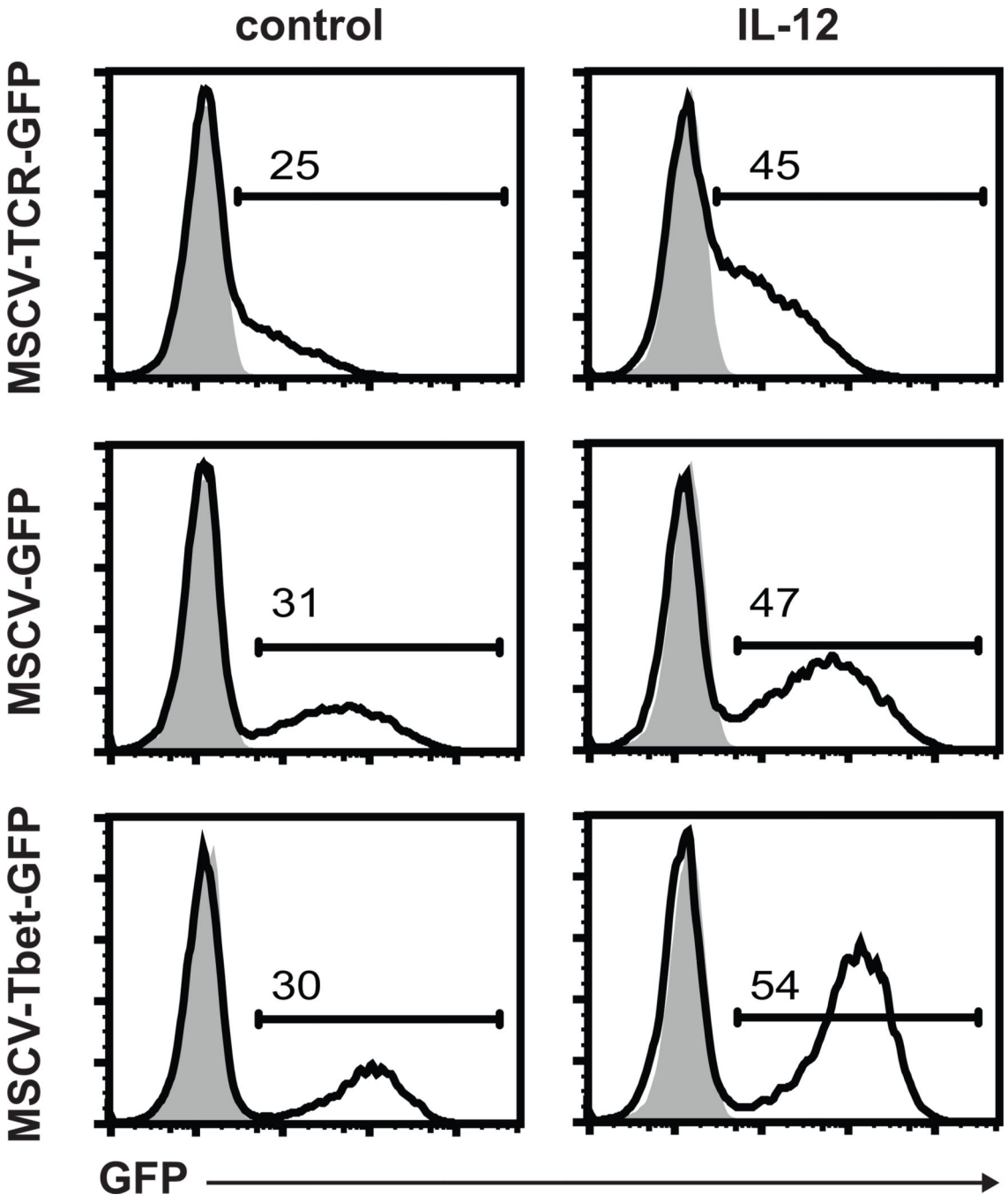

Figure 3. IL-12 conditioning during TCR stimulation enhances the transduction efficiency of mouse T cells transduced with different retroviral vectors

T cells were transduced with 3 different vectors (Tyr-TCR/s39TK-GFP, MSCV-GFP, MSCV-Tbet/GFP) as outlined in Figure 1 and assayed for GFP expression on day seven. The black line shows transduced T cells, and the shaded histogram shows control untransduced T cells. The number shows the percentage of GFP^+ cells.

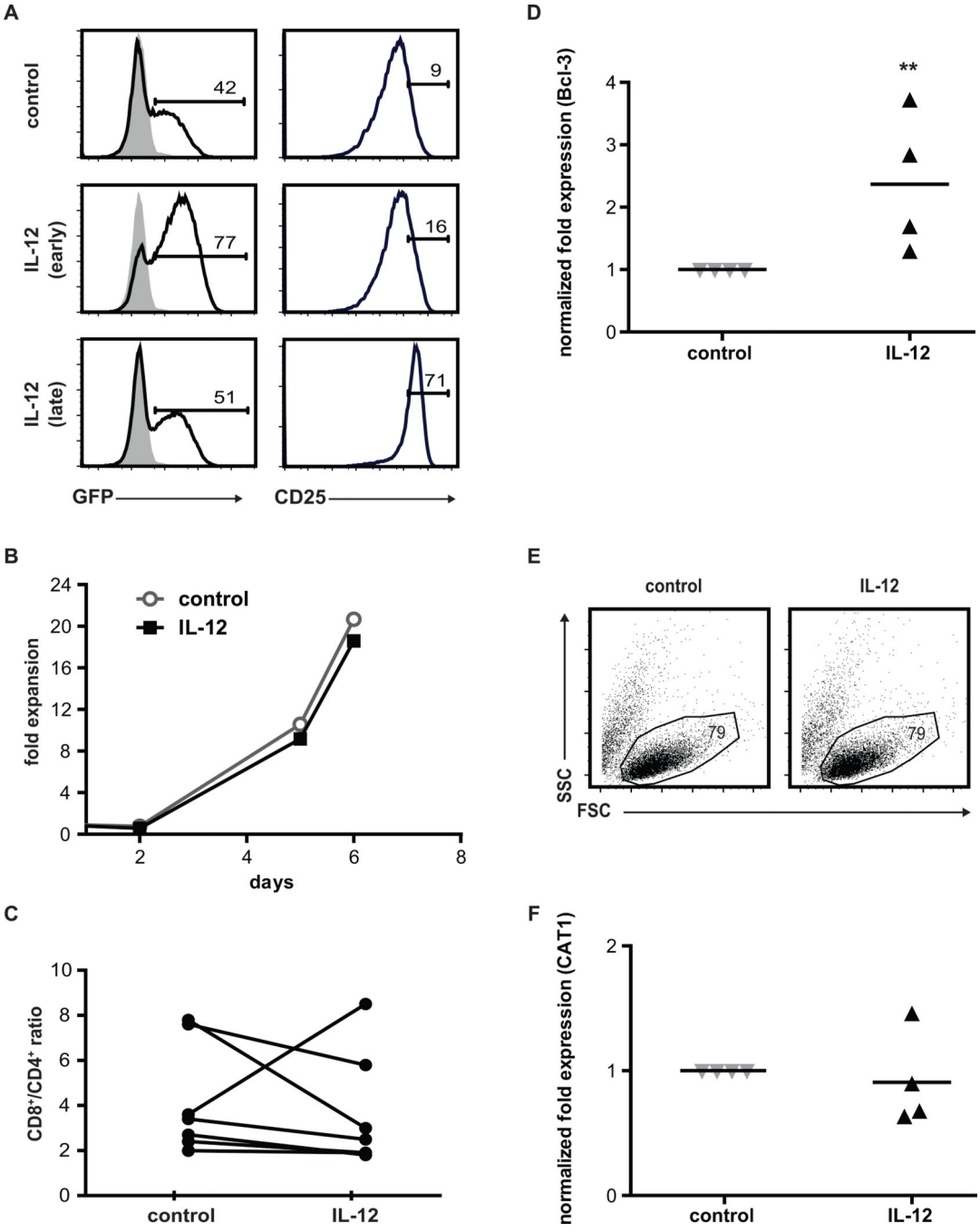

Figure 4. IL-12 conditioning during activation improves transduction efficiency without impairing cellular expansion

(A) T cells were activated as described in Figure 1 and transduced with the Tyr-TCR/s39TK-GFP vector. IL-12 was added during initial priming (first 48 hours), after the retroviral transduction (days 3–7), or not added. Flow cytometry was used to measure GFP and CD25 expression. The black line shows staining on transduced T cells, and the shaded histogram shows control untransduced T cells. The number indicates the percentage of cells gating positive for GFP or CD25. (B) Shows cellular expansion of retrovirally transduced cells conditioned with or without IL-12 during the first 48 hours. (C) Shows the CD8 to CD4

ratio of retrovirally transduced cells from 7 independent experiments. Results from the same experiment are connected with a line. (D) RNA was isolated from T cells conditioned with or without IL-12 for 48 hours, and mRNA expression levels were determined by real-time PCR. For Bcl-3, there was a significant difference (**$p<0.01$) comparing IL-12 and control conditions. Each triangle represents an independent cell preparation. (E) T cells were conditioned with or without IL-12 during the first 48 hours and assayed for viability using flow cytometry (FSC/SSC). (F) As in 'D', except cells were analyzed for CAT-1 expression. There was not a significant difference.

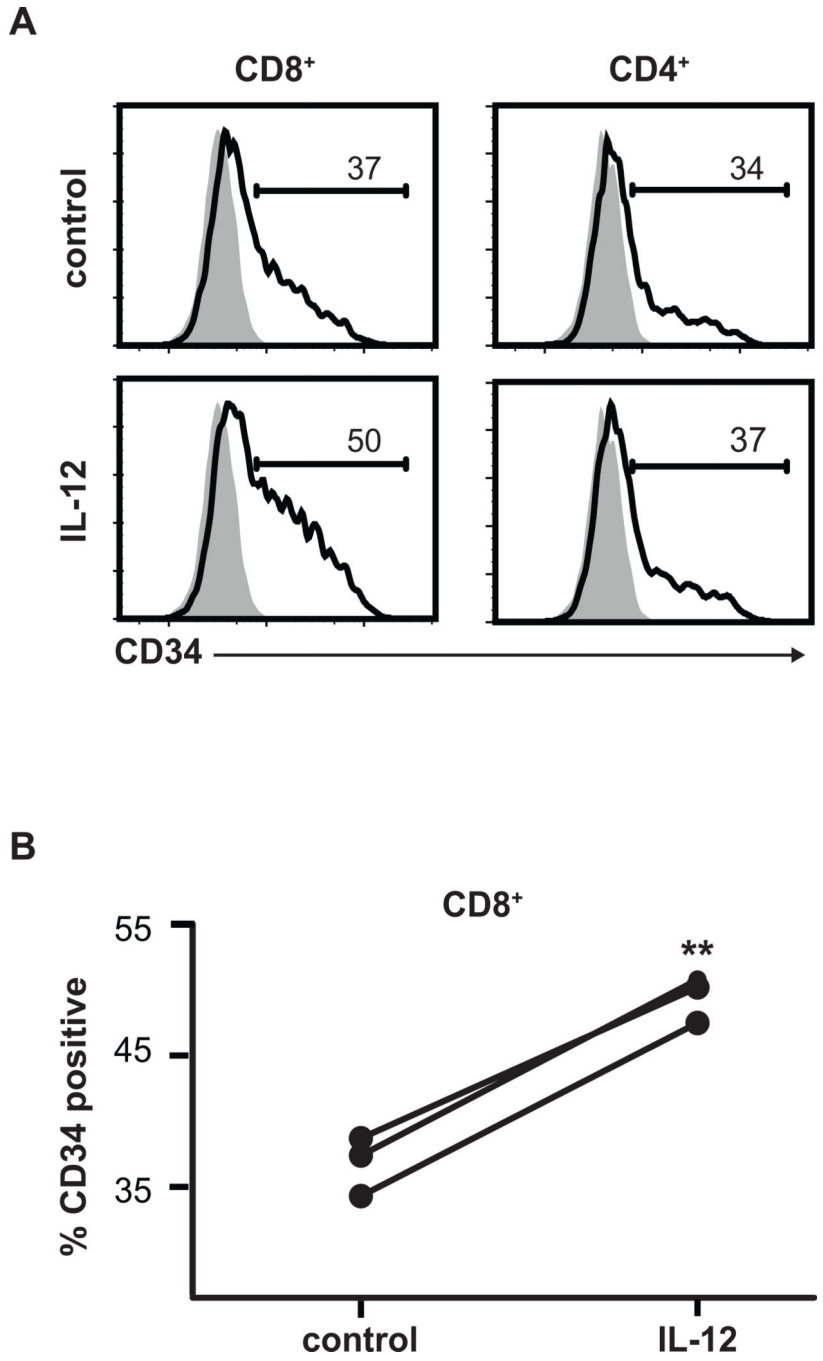

Figure 5. Human T cells conditioned with IL-12 during TCR stimulation have higher transduction efficiency

(A) Human PBMCs were stimulated with anti-CD3 mAb (OKT3) for two days with or without IL-12. On days 3 and 4, cells were transduced. On day 5, cells were analyzed by flow cytometry for CD34 expression. Shown are T cells gated for $CD8^+$ (left) or $CD4^+$ (right) expression. The black line indicates staining of transduced T cells, and the shaded histogram shows untransduced T cells stained in parallel. (B) As in 'A', except results from three independent experiments on gated $CD8^+$ human T cells are shown. The line connects points from the same experiment. The percentage indicates the frequency of transduced cells

indicated by cells expressing CD34. For B, (**) indicates a significant difference ($p<0.002$) between conditions with or without IL-12 conditioning.