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Long-Term Functionality of TCR-Transduced T Cells In Vivo

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To broaden the applicability of adoptive T cell therapy to cancer types for which tumor-specific T cells cannot routinely be isolated, an effort has been made to develop the transfer therapy of tumor-specific TCR genes into autologous T cells as a novel immunotherapeutic approach. Although such TCR-modified T cells have been shown to react to Ag encounter and can be used to break tolerance to defined self-Ags, the persistence and capacity for renewed expansion of TCR-modified T cells has not been analyzed. To establish whether TCR-transduced T cells can provide recipients with long-term Ag-specific immune protection, we analyzed long-term function of TCR transduced T cells in mouse model systems. We demonstrate that polyclonal populations of T cells transduced with a class I restricted OVA-specific TCR are able to persist in vivo and respond upon re-encounter of cognate Ag as assessed by both proliferation and cytotytic capacity. These experiments indicate that TCR gene transfer can be used to generate long-term Ag-specific T cell responses and provide a useful model system to assess the factors that can promote high-level persistence of TCR-modified T cells. The Journal of Immunology, 2008, 180: 6536–6543.

Adoptive cell therapy (ACT) of tumor-infiltrating lymphocytes (TIL) in combination with nonmyeloablative chemotherapy has been the major success of immunotherapy for melanoma in recent years (1, 2). Importantly, in a proportion of patients, substantial numbers of the adoptively transferred cells remain detectable for periods of months and retain antitumor reactivity. Furthermore, a strong persistence of the adoptively transferred T cells in the months following transfer has been shown to correlate with tumour regression (3). These data suggest that T cell-based therapies should aim for conditions where therapy-induced reactivity is not transient, but persists for prolonged periods of time.

Although the clinical results obtained with ACT for melanoma have been impressive, TIL cultures that can be used for infusion cannot be obtained for all melanoma patients. More importantly, for essentially all other human cancers, the routine production of highly tumor-reactive TIL cultures has not been successful. With the aim to develop protocols for adoptive therapy that do not rely on the availability of TIL, it has been proposed to provide autologous T cells with tumor cell-specificity, by genetic introduction of a tumor-specific TCR (4, 5). The in vivo function of TCR modified T cells has been studied extensively in mouse models. Data obtained in these studies indicate that both cytotoxic (6) and helper T cell compartments (7, 8) with a defined Ag reactivity can be generated and these cells can expand to high numbers upon in vivo Ag encounter (6, 9). Furthermore, infusion of TCR-modified T cells can be used to circumvent self-tolerance to defined self-Ags (9), including Ags expressed in spontaneous tumor models (M. de Witte, G. Bendle, M. van den Boom, M. Coccoris, T. Schell, S. Tevethia, E. Mesman, J. Song, and T. Schumacher, submitted for publication). Finally, a recent phase I clinical trial has demonstrated that TCR gene transfer is feasible in a clinical setting (10). In this trial, clinical responses were relatively rare and correlated with persistence of the TCR modified T cells. However, whether the limited persistence seen in the majority of patients is an intrinsic limitation of TCR modified T cells is unknown.

In the adoptive cell transfer protocol developed by the Rosenberg group that is based on the use of ex vivo expanded tumor-infiltrating lymphocytes, in vivo persistence of the infused cells correlates with telomere length and capacity for CD27 expression (11, 12). These data suggest that in this protocol, in vivo T cell persistence is primarily controlled by the proliferative capacity of the infused cell population. Based on these results, and on analogous data in mouse models (13), the development of culture conditions that yield tumor-specific T cells with high proliferative potential has become a major goal in ACT.

Importantly, besides the proliferative potential of the infused cells, additional factors may influence T cell persistence in case of TCR gene-modified T cells. The retroviral introduction of a tumor-specific TCR may lead to the recognition of the adoptively transferred cells by the host immune system. Such recognition could be based on the MHC-restricted presentation of epitopes in cryptic open reading frames in the viral vector used for gene modification. Alternatively, the nongermline encoded CDR3 segments of the introduced TCR may be recognized as foreign, either by host T cells or by host Abs (14). In particular, this second potential cause of immunogenicity would be problematic as the introduction of nongermline encoded TCR sequences is inherent to the strategy. In addition to the potential immunogenicity of the infused cells, the ex vivo retroviral modification of T cells may affect their capacity to form T cell memory. Before retroviral transduction, T cells are activated nonspecifically by either anti-CD3 Abs or lectins to allow retroviral gene delivery. However, the costimulatory signals

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via accessory molecules such as CD28, CD27, and 4-1BB that naive T cells receive from APCs during in vivo T cell activation are likely lacking during the in vitro activation steps used in retroviral transduction procedures. Furthermore, CD4 T cell help has been reported to be important for the development of a functional CD8 T cell memory cell pool, possibly through the induction of IL-2 expression (15–18), and it is unclear to what extent the in vitro T cell activation conditions used for retroviral T cell modification can provide these signals. Finally, arguably the greatest value of infusion of TCR gene-modified T cells lies in the treatment of human malignant disease in cases where an endogenous tumor-specific T cell repertoire is lacking due to immunological self-tolerance. In such cases, the function of infused TCR-modified T cells specific for self-Ags may be hampered by regulatory T cells, or TCR-modified T cells may become tolerized during Ag encounter in vivo.

In view of the apparent value of long-term persistence of the tumor-specific T cell response induced by ACT, in this study, we have assessed the long-term functionality of TCR-modified T cells in both nontolerant and self-tolerant settings.

Materials and Methods

Mice

C57BL/6 (B6) mice, C57BL/6L.Y5.1/5.2 (B6.L/Y5.1/5.2) F1 mice, OT-I mice (20), and RIP-OVA<sup>high</sup> mice (21) were bred in the Experimental Animal Department of The Netherlands Cancer Institute (Amsterdam, The Netherlands). All experiments were performed in accordance with institutional and national guidelines and were approved by the Experimental Animal Committee of The Netherlands Cancer Institute.

Virus infections

For live virus infections, anesthetized mice were infected by intranasal administration of 50 μl HBBS (Life Technologies) containing 200 PFU of A/WSN-33-OVA virus (WSN-OVA, Inflova) (22). For infections with vaccine recombinant for GFP-OVA<sub>237-264</sub> (rVV-OVA) (23) mice were injected i.p. with 2 × 10<sup>5</sup> PFU.

Isolation and retroviral transduction of T cells

Splenocytes were harvested from donor mice and single-cell suspensions were prepared by transferring cell suspensions through a nylon filter (NPBL, Emmer-Compascuum). Erythrocytes were lysed by NH<sub>4</sub>Cl treatment and the remaining cells were washed. For retroviral transductions, total mouse splenocytes were cultured in 24-well plates (3 × 10<sup>6</sup> cells per well) for 48 h in RPMI 1640 medium (Life Technologies) supplemented with 8% FCS (BioWhittaker), penicillin (100 U/ml), and streptomycin (100 μg/ml) (Roche Diagnostics) in the presence of Con A (2 μg/ml) (Calbiochem) and IL-7 (1 μg/ml) (Santa Cruz Biotechnology) before transduction.

Retroviral supernatants were obtained by transfection of pMX-OT-IRES-OT-I<b>h</b> TCR DNA (24) or the pMX-barcode library (K. Schepers, E. Swart, J. van Heijst, C. Gerlach, M. Castrucci, D. Sie, M. Heimeriks, A. Velds, R. Kerkhoven, and R. Arens, submitted for publication). Transductions were performed under conditions in which each TCR-transduced T cell contains approximately one barcode. In short, B6 splenocytes were retrovirally transduced with the OT-I TCR and a pmX barcode library that encodes a GFP marker gene. Transduced cells were sorted for expression of GFP, V<sub>α</sub>2 and CD8. Individual mice were injected with a mixture of 2 × 10<sup>5</sup> barcode labeled OT-I transduced T cells and 1 × 10<sup>5</sup> nonlabeled OT-I transduced T cells. After induction of primary, or primary and secondary in vivo expansion, T cells were harvested from spleen suspensions. Subsequently, barcodes were recovered by PCR and labeled with Cy3 and Cy5 dyes, and the diversity of T cell populations was determined by hybridization on barcode microarrays (K. Schepers, E. Swart, J. van Heijst, C. Gerlach, M. Castrucci, D. Sie, M. Heimeriks, A. Velds, R. Kerkhoven, and R. Arens, submitted for publication). To quantify the number of participating T cell clones, the number of barcodes with a signal above background with a probability of <0.01 was determined (K. Schepers, E. Swart, J. van Heijst, C. Gerlach, M. Castrucci, D. Sie, M. Heimeriks, A. Velds, R. Kerkhoven, and R. Arens, submitted for publication). The cut-offs used for this quantification are indicated by the horizontal and vertical dividers in the dot plots.

Results

In vivo persistence and long-term reactivity of OT-I TCR transduced T cells

In prior studies, the in vivo behavior of TCR-modified T cells has been examined in settings where the TCR-modified T cells received antigenic stimulation directly after infusion. However, in settings of minimal residual disease the capacity of infused TCR-modified T cells to persist in vivo without antigenic stimulation is likely to be important. To determine the capacity of TCR-transduced T cells to persist in vivo without antigenic stimulation is likely to be important. To determine the capacity of TCR-transduced T cells for long-term in vivo persistence without Ag encounter, B6 splenocytes (LY5.2<sup>+</sup>) were retrovirally transduced with the chicken OVA<sub>237-264</sub>–specific OT-I TCR and were injected into naive B6L.Y5.1/5.2 F<sub>1</sub> mice. In all experiments, relatively low numbers of TCR-transduced cells were given (1 × 10<sup>5</sup>), to allow extrapolation of the data toward clinically realistic protocols. Following adoptive transfer, mice were given a viral challenge with WSN-OVA, a recombinant influenza virus encoding the OVA<sub>237-264</sub> epitope, at 0, 3, 10, or 26 wk after adoptive transfer. Following
viral challenge, expansion of the gene-modified T cells in peripheral blood was measured using mAbs against Ly5.1, CD8, and TCR Vα2 and Vβ5. Ag-driven expansion of TCR-modified T cells was observed both in mice challenged with Ag directly after adoptive transfer and in mice that had received T cell infusions between 3 wk and 6 mo before challenge (Fig. 1A). Remarkably, rather than the reduction in the magnitude of T cell responses that would be expected if T cell persistence was poor, there was a trend toward more rapid and pronounced T cell responses over time, in particular in the groups of mice that were challenged at 10 and 26 wk post transfer (average T cell responses at day 7 post infection: 0 wks: 18%, 26 wks: 26%, p = 0.042) Notably, three of five mice challenged at 26 wk post infusion had to be euthanized before completion of the experiment because of severe pulmonary distress, at which time point the experiment was terminated. As prior data have demonstrated that high-level cytotoxic T cell responses against influenza A epitopes can be fatal due to T cell mediated pulmonary damage (26, M. Coccoris, unpublished data), these data seemed consistent with an increasing rather than a lessening proliferative capacity of TCR-modified T cells upon prolonged in vivo presence. To test the possibility that T cell responses mounted by TCR-modified T cells become more pronounced after prolonged in vivo presence in an independent system, mice were infused with OT-I TCR modified T cells and challenged at 0 or 6 mo with rVV-OVA. Even though OT-I-modified T cell responses in mice challenged directly after T cell infusion were mediocre in this experiment, marked T cell responses were seen in recipients that were challenged 6 mo after infusion of the cells (Fig. 1B). These findings show that without antigenic stimulation, TCR gene modified T cells can persist and remain Ag-responsive for months after cell transfer.

Secondary responses of TCR modified T cells

To subsequently address whether TCR gene-modified T cells can mount secondary responses in vivo, B6 Ly5.1/5.2 mice were injected with $1 \times 10^5$ OT-I TCR transduced T cells and mice were challenged with WSN-OVA. Six to 8 wk after primary Ag encounter, mice were rechallenged with an increased dose (1000-fold) of the identical virus. Although a prominent T cell response of the OT-I TCR modified T cells was apparent during primary Ag encounter (peak average of 17% of total CD8$^+$ cells), no significant expansion of OT-I modified T cells could be detected upon re-challenge (Fig. 2, left and middle panels). This lack of expansion of TCR-modified T cells upon secondary Ag encounter was not due to rapid clearance of WSN-OVA by pre-existing Abs, as T cells specific for the influenza A NP366–374 epitope present in the viral backbone of WSN-OVA were detected at a substantial frequency (Fig. 2, right panel). Thus, in a homologous prime-boost setting, the capacity of TCR-modified T cells to participate in a secondary T cell response was negligible. To determine whether this lack of an appreciable secondary response is an intrinsic limitation of TCR modified T cells or only observed in a homologous prime-boost setting, we evaluated secondary expansion of OT-I TCR transduced T cells upon primary challenge with rVV-OVA and rechallenge with WSN-OVA. In this heterologous prime-boost regimen, a clear expansion of OT-I TCR modified T cells is observed during secondary infection and the kinetics of expansion of the TCR transduced T cells upon secondary infection (rVV-OVA → WSN-OVA) are similar to those observed upon primary WSN-OVA infection (Figs. 1 and 3A). Notably, robust responses of TCR-modified T cells are also seen when mice receive a heterologous viral challenge 1 year or 18 mo after the primary infection (Fig. 3B).

The above data indicate that TCR gene-modified T cells that are specific for a foreign Ag can persist and participate in secondary T cell responses in vivo. However, TCR gene therapy is primarily developed as a strategy to generate a T cell repertoire that is reactive with defined self-Ags. To establish whether TCR-modified T cells that are specific for a self-Ag can also form a long-lived Ag-responsive T cell population, we examined secondary Ag responsiveness of OT-I TCR-transduced T cells in RIP-OVA$^{high}$
mice. In these mice, OVA is expressed in pancreatic β cells and, as a consequence, endogenous CD8 T cell responses against the OVA257–264 epitope are below the level of detection (9). RIP-OVA mice were infused with OT-I TCR-transduced T cells and infected with rVV-OVA (Fig. 4A). Six to 8 wk after the primary response, mice were rechallenged with WSN-OVA and OT-I modified T cell responses in peripheral blood were monitored. Using this heterologous prime-boost strategy, a robust expansion of OT-I modified T cells was observed upon re-challenge and this expansion was comparable to that observed in WT mice (Fig. 3A and 4A). These data indicate that, at least in this mouse model, TCR gene modified T cells that are directed against an Ag for which an endogenous repertoire is lacking can persist in vivo and react to secondary Ag encounter.

Dissecting the effect of mitogenic stimulation and TCR gene transfer on primary and secondary Ag responsiveness in vivo

Memory T cell responses are generally described as being more rapid and of a higher magnitude. However, the kinetics and magnitude of the secondary T cell responses of TCR-modified T cells (Figs. 3A and 4A) are comparable to those observed upon primary Ag encounter of the same recombinant virus (Fig. 1A). The disparity between these data and the generally held view on primary

**FIGURE 2.** Absence of secondary OT-I-transduced T cell responses upon homologous viral challenge. B6Ly5.1/5.2 mice (n = 5) received infusions of 1 × 10⁶ OT-I TCR transduced B6 (Ly5.2) splenocytes and were subsequently challenged by WSN-OVA infection. Six to 8 wk after primary infection, mice were rechallenged by infection with a 1000-fold increased dose of WSN-OVA. T cell responses were measured in peripheral blood samples taken at the indicated time points post viral challenge. Data represent group averages ± SD.}

**FIGURE 3.** Secondary OT-I-transduced T cell responses upon heterologous viral challenge. A, B6Ly5.1/5.2 mice (n = 5) received infusions of 1 × 10⁶ OT-I TCR-transduced B6 (Ly5.2) splenocytes and were subsequently challenged by rVV-OVA infection. Six to 8 wk after primary infection, mice were rechallenged by infection with WSN-OVA. T cell responses were measured in peripheral blood samples taken at the indicated time points post primary (left) or secondary (right) challenge. Data represent group averages ± SD. B, B6 Ly5.1/5.2 mice received infusions of 1 × 10⁶ OT-I TCR-transduced B6 (Ly5.2) splenocytes. Mice were infected with rVV-OVA directly post T cell infusion (n = 5, left panel) or at wk 26 post cell infusion (n = 7, right panel). At 78 wk post T cell infusion, a rechallenge with WSN-OVA was given. Depicted are peripheral blood T cell responses upon secondary infection. Data represent group averages ± SD.
and memory T cell responses could be due to several factors. First, the nonspecific T cell stimulation that is used for retroviral gene transfer may limit the capacity of these T cells to form T cell memory. Second, current TCR gene transfer procedures are based on the modification of pre-existing T cells that express endogenous TCR chains. The reduced expression of the introduced TCR or the formation of mixed dimers could possibly limit the competitive ability of TCR modified T cells to survive during the memory phase, or to mount a secondary response. Third, the observation that primary and secondary responses of TCR modified T cells are comparable in kinetics and magnitude may not so much reflect a reduced ability to mount secondary responses but rather an increased ability to mount rapid and strong primary T cell responses.

To address these issues, we compared primary and secondary responses of TCR modified T cells to those of naive OT-I transgenic T cells and Con A stimulated OT-I transgenic T cells (Figs. 4A, 4B). Primary T cell responses were somewhat reduced in recipients of Con A activated OT-I transgenic T cells as compared with naive OT-I transgenic T cells (average peak primary T cell responses of 19 and 27% for recipients of activated and naive T cells respectively, p = 0.014). However, secondary T cell responses were comparable between the two groups (average peak secondary T cell responses of 17% for both recipients of naive and activated OT-I transgenic T cells). Importantly, the kinetics and magnitude of secondary responses in recipients of TCR transgenic T cells were comparable to those observed in recipients of TCR-transduced T cells (Figs. 1, 4A, and 4B). These data indicate that the capacity of TCR-modified T cells for long-term persistence after Ag challenge is similar to that of unmanipulated TCR transgenic T cells. Consequently, both the in vitro activation procedure and the fact that TCR-modified T cells harbor both endogenous and exogenous TCR chains appear to have a minimal effect on their capacity for long-term and repetitive in vivo function.

**In vivo functionality of TCR-transduced T cells during secondary expansion**

The functionality of TCR-modified T cells during primary T cell responses in RIP-OVA high mice can be assessed by analyzing the development of type I diabetes as a consequence of destruction of pancreatic β cells (9). Primary T cell responses in recipients of both TCR transgenic and TCR modified T cells resulted in destruction of insulin producing cells in 100% of the mice (data not shown). As the development of type I diabetes is irreversible in this model, the use of this parameter as a read-out for T cell function during secondary responses is precluded. To assess the functionality of TCR-transduced T cells during secondary Ag-specific expansion, we therefore analyzed their capacity to destruct Ag-loaded target cells in vivo. To this purpose, a 1:1 mixture of unpulsed CFSE low RIP-OVA high splenocytes and Ova peptide pulsed CFSE high RIP-OVA high splenocytes was injected at the peak of the secondary T cell response. Six hours after injection, spleen cells were isolated and the ratio of CFSE low and CFSE high cells was...
determined (Fig. 5A). As expected, no specific killing of Ova-pulsed cells was observed in naive RIP-OVAhigh mice (average specific lysis 0%, \( n = 1005 \)). In contrast, in recipients of TCR-modified T cells that were rechallenged with Ag 6–8 wk after the primary response, efficient lysis of Ova-loaded target cells was observed (average specific lysis 43%, \( n = 1005 \)) (Fig. 5B). These data indicate that TCR-modified T cells that respond to secondary Ag challenge in a self-tolerant setting are functional, as revealed by in vivo kill of Ag-positive target cells.

Secondary responses of TCR-modified T cells are polyclonal

To determine whether the adoptively transferred T cells participating in a secondary response are polyclonal or derived from a limited set of T cells, we labeled OT-I TCR-transduced T cells with unique identifiers (“barcodes”) by retroviral infection. To this purpose, we made use of a retroviral plasmid library, containing 3,000 unique sequences, all coupled to a GFP marker gene (K. Schepers, E. Swart, J. van Heijst, C. Gerlach, M. Castrucci, D. Sie, M. Heimerikx, A. Velds, R. Kerkhoven, and R. Arens, submitted for publication). After adoptive transfer of 2\( \times 10^3 \) barcode-labeled TCR transduced T cells plus 1\( \times 10^5 \) non-barcode-labeled TCR-transduced T cells, recipient mice were challenged with rVV-OVA. Analysis of barcode diversity was subsequently performed at the peak of the primary response for one group of mice (Fig. 6A, left panel). A second group of mice was rechallenged with WSN-OVA 6 wk later and barcode diversity was determined at the peak of the secondary T cell response (Fig. 6A, right panel). When barcode diversity is compared between primary and secondary T cell responses, the number of clones with detectable participation is essentially identical. Thus, secondary T cell responses of TCR-modified T cells remain highly polyclonal and are not driven by expansion of one or a few T cell clones (Fig. 6B).

**Discussion**

TCR gene transfer allows the generation of T cells with a defined Ag specificity, even when this specificity is lacking in the endogenous T cell repertoire. This strategy can be considered analogous...
to the clinical use of Abs such as Herceptin and Rituxan that recognize tumor-associated self-Ags for which the human immune system is tolerant. However, while a substantial amount of data has been generated on the pharmacokinetics of therapeutic Abs, no data is available on the in vivo persistence and maintenance of function of TCR-modified T cells.

A number of issues may conceivably limit the in vivo persistence of TCR-modified T cells. After adoptive transfer, the infused gene-modified T cells may be recognized and targeted by the immune system of the recipient. Alternatively, the long-term function of TCR-modified T cells may be reduced as a consequence of the retroviral modification procedure. Clinical studies using TIL infusion have demonstrated that the long-term persistence of adoptively transferred cells correlates with clinical responses (3). As a durable engraftment of TCR-modified T cells is likewise expected to be required, in this study, we examined the function of TCR-modified cells for up to 18 mo after adoptive transfer. In these experiments, mice were infused with \( 1 \times 10^7 \) TCR-modified T cells, the equivalent of \( 1 \times 10^9 \) TCR-modified T cells in humans (assuming a CD8\(^+\) T cell pool of \( 2 \times 10^7 \) and \( 2 \times 10^4 \) in mice and humans, respectively), slightly below the average dose used by Morgan et al. (10). The two main conclusions from these experiments are that retrovirally TCR-transduced T cells can persist for prolonged periods in vivo without a requirement for Ag-specific stimulation and that TCR-modified T cells provide a capacity for secondary T cell responses that is comparable to that of nonmanipulated TCR transgenic cells.

Ag-reactive TCR-modified T cells persisted for periods of at least 18 mo after adoptive transfer in the current experiments. This indicates that, at least in this system, the infused cells are not rendered immunogenic by TCR gene modification and that the retroviral activation procedure does not impair the capacity of TCR-modified T cells for long-term engraftment. A further concern in TCR gene transfer has focused on the possibly of limited stability of TCR transgenes in gene modified T cells. Specifically, mouse studies have shown that silencing of retroviral transgenes in hematopoietic cell types may occur (27). Furthermore, transcription from the retroviral LTR was shown to be reduced in quiescent T cells (28–29). The current data demonstrate that, at least for murine T cells, such inactivation of transgene expression is insufficiently strong or frequent to prevent robust T cell responses of TCR-modified T cells upon introduction of Ag at later time points. By examining clonal diversity in TCR-modified T cell responses, it was also shown that secondary responses of TCR-modified T cells are not driven by a limited set of clones. These data provide some evidence that retroviral modification of T cells may not lead to the preferential outgrowth of cells that carry integrations near genes controlling proliferative capacity that has been observed for gene-modified hematopoietic stem cells (30). In this regard, it is also interesting to note that in mice that received OT-I modified T cells, no rise in the frequency of V\( \alpha 2^+ V\beta 5^+ \) T cells was seen during a follow-up of 18 mo, unless Ag was provided (Fig. 3B, data not shown). These data suggest that in this murine model, the risk for cellular transformation due to retroviral modification with TCR-encoding vectors may be low. However, studies with a longer follow-up will clearly be required.

Interestingly, the magnitude of TCR-modified T cell responses appears to increase upon prolonged periods of in vivo persistence. The in vitro activation of T cells that is required for retroviral gene transfer leads to phenotypic maturation, including down-regulation of CD62L expression. As a consequence, TCR-modified T cells may have a reduced capacity for lymph node entry, thereby reducing Ag responsiveness shortly after infusion (31). Conceivably, acquisition of a central memory T cell phenotype, including reduced expression of CD62L, could then explain the enhanced responsiveness of the infused cells over time (32).

In a second set of experiments, it was demonstrated that TCR-modified T cells have the capacity to react to secondary Ag encounter in vivo. To achieve a productive secondary response of TCR-modified T cells, it was essential to use a heterologous prime-boost regime. This indicates that, even in a setting where the T cell repertoire is highly biased at the start of the primary response, immunodominance of other T cell specificities (33) can interfere with activation of the infused cell population during secondary Ag encounter. Notably, the kinetics and magnitude of secondary T cell responses obtained in these prime-boost experiments were not increased as compared with primary T cell responses of the same TCR-modified T cells. This contrasts with the classical description of memory T cell responses, typically characterized by an accelerated expansion and increased magnitude (34, 35). Importantly, this difference with classical memory T cell responses is not due to the process of TCR introduction, as the same phenomenon is observed after infusion of nonmodified TCR transgenic T cells (Fig. 4). Thus, the observed lack of increased T cell responsiveness in recall responses is likely due to the fact that a large T cell pool is already available for Ag recognition at the start of the primary T cell response, as a consequence of the adoptive transfer.

Finally, to analyze a possible effect of the TCR gene transfer procedure on the capacity for Ag-specific proliferation of the adoptively transfered cells, we compared responsiveness of TCR transduced cells with that of quiescent or activated TCR transgenic T cells with the identical specificity. Results of these experiments show that primary T cell responses of TCR modified T cells are some 2–3-fold reduced as compared with those of naive TCR transgenic cells. As discussed above, this may at least in part be due to a detrimental effect of the in vitro T cell activation procedure on the ability of cells to participate in the Ag-specific T cell response. In line with this, primary (but not secondary) T cell responses of TCR transgenic T cells are also reduced to some extent by in vitro T cell activation. These data provide indirect support for the preclinical and clinical testing of lentiviral vector systems that do not require mitogenic T cell stimulation.

In conclusion, the current data demonstrate that TCR transduced T cells remain present in the host after adoptive transfer and can be activated in vivo up to 18 mo after infusion. Furthermore, the capacity of TCR gene-modified T cells to react upon secondary Ag encounter in vivo, is comparable to that of T cells that have not undergone in vitro modification. These features, together with the previously described capacity for recognition of defined tumor-associated self-Ags, provide a clear incentive for the further clinical testing of TCR modified T cells in oncology.

Disclosures
The authors have no financial conflict of interest.

References


