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TCR Gene Therapy of Spontaneous Prostate Carcinoma Requires In Vivo T Cell Activation

Moniek A. de Witte,2,‡ Gavin M. Bendle,2,‡ Marly D. van den Boom,* Miriam Coccoris,2,‡ Todd D. Schell,§ Satvir S. Tevethia,§ Harm van Tinteren,‡ Elly M. Mesman,† Ji-Ying Song,† and Ton N. M. Schumacher4,*

Analogous to the clinical use of recombinant high-affinity Abs, transfer of TCR genes may be used to create a T cell compartment specific for self-Ags to which the endogenous T cell repertoire is immune tolerant. In this study, we show in a spontaneous prostate carcinoma model that the combination of vaccination with adoptive transfer of small numbers of T cells that are genetically modified with a tumor-specific TCR results in a marked suppression of tumor development, even though both treatments are by themselves without effect. These results demonstrate the value of TCR gene transfer to target otherwise nonimmunogenic tumor-associated self-Ags provided that adoptive transfer occurs under conditions that allow in vivo expansion of the TCR-modified T cells. The Journal of Immunology, 2008, 181: 2563–2571.

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The shared tumor-associated Ags (TAAs)1 that are potential targets of cancer immunotherapy primarily consist of nonmutated self-Ags that are either lineage-specific or overexpressed. Due to immunological tolerance toward these proteins, B cells producing high affinity Abs and T cells expressing high-affinity TCRs are often deleted, making the remaining repertoire relatively unresponsive to active immunization. Furthermore, for those Ags for which self-tolerance is incomplete, the process of tumor development can actively tolerize the remaining T cells (1). In the absence of an effective endogenous TAA-specific immune repertoire, passive immunization with TAA-specific Abs or T cells may be considered a preferred approach (2). In line with this, the clinical use of recombinant Abs such as Rituximab that targets CD20 and Trastuzumab that targets Her2/Neu has been a major advance in the treatment of human cancer over the past decade (3).

Analogous to the transfer of high-affinity Abs, adoptive transfer of exogenous tumor-specific TCRs into endogenous T cells (a process hereafter referred to as TCR gene transfer) might be used to generate T cells directed toward TAAs such as wilms tumor Ag 1 (WT-1) and Preferentially Expressed Ag of Melanoma (PRAME). Like the HER-2/neu protein targeted by Trastuzumab, these Ags are nonmutated proteins that are over expressed by cancer cells, being either leukemic (4) or melanoma cells (5). Furthermore, for all these proteins, over-expression contributes to cellular transformation, making tumor escape a less likely event (6–8). In the present study, we set out to analyze the value of TCR gene transfer in the targeting of this class of tumor-associated Ags in a murine spontaneous tumor model. These experiments assess the feasibility of TCR gene therapy for tumor types for which classical forms of adoptive T cell therapy may be precluded and compare it to the value of active vaccination.

Materials and Methods

Mice

C57BL/6 (B6) and transgenic adenocarcinoma of mouse prostate (TRAMP) mice (9) were obtained from the Experimental Animal Department of The Netherlands Cancer Institute. For all experiments, F1 offspring of B6 × TRAMP mice was used. All animal experiments were performed in accordance with institutional and national guidelines and were approved by the Experimental Animal Committee of The Netherlands Cancer Institute.

Retroviral constructs and retroviral transduction of T cells

The SV40IgV specific TCR α- and β-chains were cloned by standard procedures from the cytotoxic T lymphocyte clone Y-4. The Y-4 clone was isolated by limiting dilution from CTL-derived from B6 mice immunized with SV40 large T Ag transformed syngeneic kidney cells and has been described previously (10). A pMX-SV40α-RES-SV40β vector was generated and used to transfect Phoenix-E packaging cells to generate retrovirus (11). Mouse splenocytes were modified by retroviral transduction as described previously (12).

Flow cytometry

Surface TCR expression was measured 24 h post transduction by flow cytometry. Cells were stained with FITC-labeled anti-TCR V/β 9mAb and PE-labeled anti-TCR V/β 2, 3, 4, 5.1, 8, 11, and 10b mAb (anti V/β-pool), or with MHC tetramers, in combination with PE- or allophycocyanin-conjugated anti-CD8α mAb from Caltag). Propidium iodide (Sigma-Aldrich) was used to select for live cells. For the measurement of Ag-specific T cell responses in peripheral blood, samples were taken at the indicated days post transfer. Following removal of erythrocytes by NH4Cl treatment, cells
were stained with the above-mentioned Abs and analyzed by flow cytometry. For analysis of SV40-specific T cell responses in spleen and prostate, mice were sacrificed 11 days post vaccination, spleen and prostate tissue were harvested, and single cell suspensions were obtained by macerating tissues through a 40-µm nylon cell strainer. Intracellular IFN-γ staining was performed as previously described (12). Data acquisition and analysis was done on a FACSCalibur (Becton Dickinson) with FCS Express software (De Novo Software) or CellQuest Pro software (BD Biosciences).

Viral infection
For live influenza A infections, anesthetized mice were infected by intranasal administration of 50 µl of HBSS (Life Technologies) containing 1000 PFU of influenza A/WSN/33 (WSN)-SV40(IV) virus (flu-T) (K. Schepers, E. Swart, and T. Schumacher, unpublished data). For vaccinia infections, 1 × 10^5 PFU rVV-ES-IV (rVV-T) was injected i.p (13).

Histopathology
Tissues were sampled in buffered formalin and stained with H&E. The sections were reviewed with a Zeiss Axioskop2 Plus microscope (Carl Zeiss Microscopy, Jena, Germany) equipped with Plan-Apochromat (×5/0.16, ×10/0.45, ×20/0.60, and ×40/0.95) and Plan-Neofluar (×2.5/0.075) objectives. In addition to the objectives, there was an extra enlargement device included in the body of the microscope. Images were captured with a Zeiss AxioCam HRc digital camera and processed with AxioVision 4 software (both from Carl Zeiss Vision). Pathological examination and classification of the prostate gland, coagulation gland, and seminal vesicles was performed blindly, according to The Consensus Report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium (14).

Immunohistochemistry
Immunohistochemistry was conducted on buffered formalin sections. Sections were preincubated with PBS/4% BSA. For large T immunostaining, sections were stained for 1 h with SV40 pb 101 primary Ab (1/500, Becton Dickinson), followed by a two-step immunoenzymatic procedure. First, biotin labeled goat-anti-mouse immunoglobulins (DakoCytomation, 1/500, 1 h) were applied, followed by HRP-labeled avidin-biotin complex (DakoCytomation, 1 h). AEC (Sigma-Aldrich) was used as a substrate-chromagen and slides were counterstained with hematoxylin. Images were acquired using an Axioacam HR digital camera and processed with Axiovision 4 software (Carl Zeiss Vision GmbH).

Statistical analysis
To test whether two treatments had the same underlying multinomial (ordered) distribution of type of pathology, a Stratified Wilcoxon-Mann-Whitney U test for ordered categorical responses was used. Two-sided p values are reported.

Results
Development of neoplastic lesions and SV40 expression in TRAMP mice
TRAMP (transgenic adenocarcinoma of mouse prostate) mice express the transforming protein SV40 large T under control of the prostate specific probasin promoter, resulting in the development of prostatic intraepithelial neoplasia (PIN) lesions from 8 to 12 wk of age and carcinomas by 18 wk of age (9; see below). The epithelial cells within prostate areas that undergo pathologic alterations (ranging from atypical hyperplasia to carcinomas) display high SV40 large T expression, as evidenced by immunohistochemistry (Fig. 1, A–C). In contrast, SV40 large T expression in prostate cells with a normal morphology is generally below the limit of detection (Fig. 1, A–C).

The endogenous T cell repertoire of TRAMP mice is tolerant toward SV40IV at the time neoplastic lesions first develop
Prior work has demonstrated that young (4–7 wk) male TRAMP mice harbor a residual low avidity T cell repertoire specific for SV40IV, the immunodominant epitope of the large T oncoprotein. However, from 10 wk of age onwards, this low avidity T cell repertoire is no longer detectable (15). To corroborate these data, 11-wk old TRAMP mice and nontransgenic littermates were im-
reactivity against the SV40IV epitope was monitored by MHC tetramer staining, a sample of the transduced T cells was stained with Kb-SV40404–411 tetramers and PE-anti-CD8 Abs to determine the percentage of SV40IV-specific CD8+ cells. Post peptide stimulation, cells were stained with allophycocyanin-anti-CD8, permeabilized, and stained with PE-anti-IFN-γ. Flow cytometric analysis of blood cells of B6 (left panels) and TRAMP mice (right panels) that received 1 × 10^5 SV40IV-TCR transduced T cells (top panels) or no transfer of T cells (bottom panels) followed by an i.n. infection with 1000 PFU of flu-T. Blood was sampled 3–14 days post infection. Circles represent TCR-transduced T cell responses in individual mice; bars indicate averages.

revealed that IFN-γ producing SV40IV-specific T cells were barely detectable in TRAMP mice, but abundantly present in nontransgenic littermates (Fig. 2, A and B). Furthermore, when reactivity against the SV40IV epitope was monitored by MHC tetramer staining, SV40IV-specific T cell responses were also highly reduced in vaccinated TRAMP mice (data not shown). Consistent with prior peptide and DC vaccination studies (15, 16), these data demonstrate that the endogenous T cell repertoire of TRAMP mice is tolerant toward SV40IV at the time prostatic lesions first develop. The restricted over-expression of large T in (pre)malignant tissue, its role in transformation, and the induction of tolerance toward large T in tumor-associated self Ags such as WT-1 and PRAME.

**TCR gene transfer can be used to create a SV40IV-reactive T cell compartment that is otherwise absent in TRAMP mice**

Prior work has shown that the combination of vaccination with adoptive transfer of T cells modified with a relevant TCR, but not a control TCR, can be used to induce self Ag-specific T cell responses (17). To assess the value of the induction of self Ag-reactive T cell responses by TCR gene transfer in the targeting of developing tumors, we isolated an SV40IV-specific TCR (11) and used this TCR to modify T cells of TRAMP mice. Following retroviral transduction, on average 25–40% of CD8+ T cells expressed the SV40IV TCR, and these cells produced IFN-γ upon incubation with the relevant Ag in vitro (Fig. 3, A and B). To address whether SV40IV TCR-modified T cells could proliferate upon in vivo Ag encounter, TRAMP mice and as a control B6 mice received an adoptive transfer of 1 × 10^5 SV40IV TCR CD8+ T cells, followed by an intranasal flu-T infection. Subsequently, TCR-modified T cell responses were monitored by measuring the fraction of T cells expressing one of a set of endogenous Vβ elements together with the Vβ element used by the introduced TCR (17). Although an endogenous SV40IV-specific T cell response could not be detected in TRAMP mice (Fig. 2), SV40IV-TCR transduced T cells proliferated strongly upon Ag encounter in vivo, as assessed by the emergence of a Vβ9+Vβ-pool+ T cell population. In line with the notion that the Vβ9+Vβ-pool+ T cell population consists of TCR-modified T cells, no increase in Vβ9+Vβ-pool+ T cell numbers was seen in recipients of mock-transduced T cells (Fig. 3C, bottom panels). Frequencies of Vβ9+Vβ-pool+ T cells peaked around day 10 post transfer and subsequently decreased significantly. This contraction of the TCR-modified-specific T cell response is consistent with prior data on the kinetics of endogenous vaccine-induced influenza A-specific T cell responses (18) and is also observed for the endogenous SV40IV-specific T cell response in B6 mice (data not shown). These experiments show that TCR gene transfer can be used to create a TAA-reactive T cell compartment that is otherwise absent (Fig. 3C). Interestingly, SV40IV-TCR transduced T cell responses in TRAMP mice are on average ~1.5–2-fold lower than those in B6 controls (average peak T cell response of 8.0 vs 12.9%), suggesting that tolerizing mechanisms may have some impact on the in vivo potential of TCR-modified T cells.

**In vivo distribution and function of SV40IV TCR-modified T cells**

To examine the in vivo distribution and function of SV40IV TCR-modified T cells, prostate glands and spleen samples were isolated from TRAMP and B6 mice at day 11 post vaccination. As expected, Vβ9+Vβ-pool+ T cells were detected in spleen samples from B6 and TRAMP mice that had received SV40IV TCR-modified T cells, whereas in control mice that were only vaccinated this
population was absent (Fig. 4, A and B). Furthermore, Vβ9+/Vβ-pool- CD8+ cells were also detected in prostate samples from B6 and TRAMP mice, indicating that homing to the prostate can occur independent of Ag expression. Splenic T cells in B6 and TRAMP mice produced high levels of IFN-γ after stimulation with the SV40IV Ag. In TRAMP but not B6 mice, this production was dependent upon adoptive T cell transfer, reflecting that an endogenous SV40IV-specific T cell repertoire is lacking in TRAMP but not B6 mice. Notably, no substantial Ag-specific IFN-γ production was detected within prostate tissue, suggesting that the effector function of TCR-modified T cells may possibly be suppressed at this site (Fig. 4).

Table I. A combination of adoptively transferred SV40IV TCR-modified T cells and vaccination inhibits tumor progression in TRAMP mice

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TRAMP mice received vaccination with two SV40IV-recombinant viruses at wk 10 and 16. A second group of mice received vaccination with the same recombinant viruses plus ACT with a small number of TCR-modified T cells (5 × 10^5) at the same time points. Two weeks after the second treatment (wk 18), mice were sacrificed and analyzed for tumor development in prostate glands, coagulation glands, and seminal vesicles. Numerical values depict the number of mice with the indicated lesion type within that cohort. In cases where multiple types of lesions were detected within one organ, the most severe type was used for scoring. Numbers in bold indicate the number of mice per treatment group that were scored with the indicated lesion type.

FIGURE 4. Homing and functional properties of SV40IV-TCR modified T cells. Ten-wk-old TRAMP mice and control nontransgenic littermates received an adoptive transfer of 5 × 10^5 SV40IV-TCR transduced T cells, followed by vaccination by i.p. infection with 1 × 10^5 PFU. of rVV-T. Control mice were solely vaccinated with rVV-T. Eleven days post vaccination, the frequency of TCR transduced cells in spleen and prostate was assessed by analyzing the percentage of Vβ9+/Vβ-pool- CD8+ cells of total Vβ-pool+ CD8+ cells. Functionality of SV40IV-specific T cells was measured by intracellular IFN-γ staining after incubation for 4 h with 100 ng/ml of the relevant peptide (SV40 404-411) or control peptide (OVA257-264). Shown are dot plots from a mouse in each of the treatment groups (A) and cumulative data from all mice (B). Numbers in upper right corner of dot plots refer to percentage of Vβ9+/Vβ-pool+ cells of total CD8+ cells or IFN-γ+CD8+ cells of total CD8+ cells. Circles in graphs represent individual mice, bars indicate averages.
FIGURE 5. Adoptive transfer of SV40 IV-TCR transduced T cells results in a marked delay in tumor development. A, Outline of experiment. Ten-wk-old TRAMP mice received an adoptive transfer of 2.5–5 × 10⁵ SV40IV-TCR-transduced T cells, followed by vaccination by means of an i.p. injection with 1 × 10⁶ PFU of rVV-T. Six weeks later, the same mice received a second infusion of 2.5–5 × 10⁵ SV40IV-TCR transduced T cells, followed by i.n. injection with 1000 PFU of flu-T. Control mice were either vaccinated with the two recombinant viruses or were left untreated. Blood was sampled at various time points for 2 wk post each infusion and analyzed blindly by flow cytometry. At 28 wk, mice were sacrificed and indicated sites were analyzed for tumor development by histopathology. B, The percentage of Vβ9/VβpoolCD8⁺ cells of total VβpoolCD8⁺ cells in peripheral blood. Circles represent individual mice, bars indicate averages. Shown are results of one of two experiments. C, Classification of tumor development in prostate gland, coagulation gland, and seminal vesicles in treated and nontreated TRAMP mice. Bars depict the percentage of mice with the indicated lesion type within that cohort. In cases where multiple types of lesions were detected within one organ, the most severe type was used for scoring. Shown are the pooled results of two independent experiments, compared with pathology found in 9-wk old TRAMP mice (top row). To test for treatment effects, Wilcoxon-Mann-Whitney tests were performed, adjusting for organ type. Vaccination only vs no treatment, p = 0.9152; TCR gene transfer vs no treatment, p < 0.0001; TCR gene transfer vs vaccination only, p < 0.0001; TCR gene transfer vs the pathology found in 9-wk-old mice, p = 0.3775. D, Macroscopic analysis of the male reproductive tract of a representative TRAMP mouse of each of the three treatment groups, compared with the male reproductive tract of a 28-wk-old nontransgenic littermate. E, SV40 large T expression in prostate and coagulation gland of a 28-wk-old TRAMP mouse after two transfers of SV40IV-TCR transduced T cells in combination with viral vaccination. Dorsal prostate gland (D), lateral prostate gland (L), coagulation gland (C). Cells showing atypical hyperplasia are SV40 large T immunostaining positive (bottom panels), whereas cells with a normal morphology in the same coagulation gland are SV40 Large T immunostaining negative (left bottom panel). Original magnifications: ×2.5 (top panel) and ×40 (bottom panels).
A combination of adoptively transferred SV40 IV TCR-modified T cells and vaccination leads to the long-term suppression of tumor progression in TRAMP mice

To determine the potential impact of adoptive cell therapy (ACT) with TCR-modified T cells on tumor development, a pilot study was performed. A first group of TRAMP mice (n = 5) received vaccination with two SV40 IV-recombinant viruses at week 10 (when PIN lesions are detectable in prostate and coagulation glands in the majority of animals) and at week 16. A second group of mice received vaccination with the two recombinant viruses without ACT. C and D, Ten-week-old TRAMP mice received an adoptive transfer of 2.5–5 × 10^5 SV40 IV-TCR-transduced T cells, followed by a second infusion 6 wk later. Control mice were left untreated. A and C, Blood was sampled at the indicated time points to determine the percentage of Vβ9^+^ Vβpool^−^ CD8^+^ cells of total Vβpool^+^ CD8^+^ cells. Circles represent individual mice, bars indicate averages. B and D, At 28 wk, mice were sacrificed and indicated sites were analyzed for tumor development by histopathology. Bars depict the percentage of mice with the indicated lesion type within that cohort. In cases where multiple types of lesions were detected within one organ, the most severe type was used for scoring. To test for treatment effects, Wilcoxon-Mann-Whitney tests were performed. B: Two cycles of ACT/vaccination vs vaccination only, p < 0.001; One cycle of ACT/vaccination vs vaccination only, p < 0.01; Two cycles of ACT/vaccination vs one cycle of ACT/vaccination, p = 0.2166; D: TCR gene transfer without concomitant vaccination vs no treatment, p = 0.5055.

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(experimental setup in Fig. 5A, analysis of TCR-modified T cell responses in Fig. 5B). At week 28, mice were sacrificed to analyze tumor development in prostate glands, coagulation glands, and seminal vesicles (Fig. 5, C and D). In untreated mice, carcinomas developed in all prostate glands (100% of mice) as well as in coagulation glands (60% of mice). In the seminal vesicles, atypical hyperplasia (50% of mice) and adenomas (40% of mice) were detected. Importantly, in mice treated by vaccination only, the incidence of carcinomas in prostate glands (100% of mice) and coagulation glands (55% of mice), and the incidence of adenomas in the seminal vesicles (67% of mice) was not reduced to any measurable extent ($p = 0.9152$, Wilcoxon-Mann-Whitney U test).

However, when mice received the same vaccination protocol in combination with infusion of SV40IV-TCR-modified TRAMP T cells, the incidence of carcinomas and adenomas was substantially reduced. In both prostate and coagulation glands, only premalignant lesions were detected in the majority of mice (73 and 64% of mice, respectively). In the seminal vesicles, no abnormalities were detected in 10 of 11 mice (Fig. 5, C and D).

These data show in a spontaneous tumor model that the combination of TCR gene transfer and vaccination significantly suppresses tumor progression ($p < 0.0001$) and that this effect is long lasting (months upon ACT). Notably, the low-grade lesions that are still detected in tissue sections of mice that were treated by TCR gene therapy remain large T-positive (Fig. 5E, lower left panel). In a recent study that showed that vaccination of 8-wk-old TRAMP mice against prostate stem cell Ag can lead to the long-term suppression of tumor development, low-grade lesions were also still detectable in treated mice (19). However, in this case, the remaining lesions were largely Ag negative. To provide a possible explanation for the persistence of low-grade lesions in the current setting, in which an Ag essential for tumor growth is targeted, we analyzed SV40IV-specific T cell responses of 28 wk old TRAMP mice treated by TCR gene therapy. Interestingly, this analysis revealed that SV40IV TCR-modified T cells were barely detectable at this time point in ex vivo spleen samples and could not be expanded in vitro T cell cultures (data not shown). These data suggest that the prolonged presence of TCR-modified T cells in an Ag-bearing host may lead to deletion. Previous studies in TRAMP mice have demonstrated that both deletion and mechanisms of nondeletional tolerance can impede prostate tumor specific T cell responses (15, 20–23). Future studies will be required to determine which mechanisms of tolerance induction can impair the long-term function of adoptively transferred SV40IV TCR-modified T cells in TRAMP mice.

To directly test whether multiple T cell infusions are required for the long-term suppression of tumor progression, TRAMP mice received either a single cycle of TCR-modified T cell transfer/vaccination at week 10, or two cycles of TCR-modified T cell transfer/vaccination at week 10 and 16. In both groups of mice, tumor progression was significantly ($p < 0.01$ and $p < 0.001$ for one and two cycle groups, respectively) inhibited as compared with recipients of vaccination only. Thus, while there is a (non-significant; $p = 0.2166$) trend toward an increased antitumor effect in mice receiving two cycles of ACT, a single cycle of ACT at week 10 can greatly reduce prostate carcinoma development in the following 4 mo (Fig. 6, A and B).

Concomitant vaccination is an essential requirement for the suppression of prostate carcinoma by adoptively transferred SV40IV, TCR-modified T cells in TRAMP mice

The above data demonstrate that the combination of vaccination with TCR-modified T cell transfer suppresses tumor outgrowth. However, these experiments do not address whether vaccination is in fact required to achieve this effect. To address this issue, a group of TRAMP mice received two adoptive transfers of SV40IV, TCR-modified T cells at weeks 10 and 16 without further vaccination. Analysis of Vβ9Vβ9”Vβ9” cell responses in peripheral blood of these animals showed that frequencies of TCR-modified T cells stayed close to background in the absence of vaccination (Fig. 6C). In line with the observation that vaccination is essential to drive expansion of the TCR-modified T cell pool, tumor progression was not measurably affected by the sole transfer of TCR-modified T cells (Fig. 6D).

Discussion

Approaches for adoptive T cell therapy of human cancer can be divided into two broad categories based on the use of either allogeneic or of autologous T cells. In allogeneic T cell therapy, as exemplified by donor lymphocyte infusions for patients with chronic myeloid leukemia, the infused T cells can recognize polymorphic epitopes expressed by tumor cells as truly foreign Ags, and thus with high avidity (24). The very high response rate seen after donor lymphocyte infusions treatment (>50% for patients with relapsed chronic myeloid leukemia; Ref. 25) underscores the clinical value of T cells that can recognize tumor-associated Ags with such high avidity. However, the requirement for an allogeneic hematopoietic stem cell transplant, plus the frequent development of graft-vs-host-disease, due to T cell recognition of polymorphic Ags expressed by other cell types, form two very substantial limitations of allogeneic T cell therapy. Autologous T cell therapy, by means of infusion of ex vivo expanded patient-derived tumor infiltrating lymphocytes (TILs), has a substantial clinical effect in patients with metastatic melanoma, with an objective response rate of ~50% (26–28). Autologous T cell therapy is not complicated by the development of graft-vs-host-disease, however, it is restricted to those patient groups for which tumor-infiltrating lymphocytes can be isolated. Specifically, while melanoma-reactive T cell responses can be observed rather frequently, for most other tumor types, the detection of T cell infiltrates with a clear potential for tumor recognition is a rare event.

A comparison of the advantages and disadvantages of allogeneic and autologous T cell therapy suggests that the adoptive transfer of T cells that are autologous, but do express high affinity tumor specific TCRs would be an attractive approach, and this has been the goal of efforts to generate redirected T cells by TCR gene transfer (29). Following a series of in vitro analyses, the in vivo function of TCR gene-modified T cells has been established in mouse models (12, 17, 30, 31). Furthermore, a recent phase I clinical trial in patients with metastatic melanoma has demonstrated that the transfer of TCR modified T cells is clinically feasible and that the resultant cells show long-term engraftment in some patients (32). Although the objective response rate in this trial has been low, this study provided the first evidence that, at least for a tumor type that was previously shown to be responsive to TIL treatment, TCR gene therapy is a realistic option.

The goals of the current study were two-fold: first, to establish whether TCR gene therapy could be developed into a realistic option for tumor types for which TIL transfer is not feasible and second, to make such an assessment in a spontaneous tumor model rather than a transplantable tumor model. The latter issue is of some importance as immunogenicity of unknown neo-Ags that have been introduced or have accumulated during in vitro growth can severely complicate the interpretation of immunological interventions in transplantable tumor models (33). In addition, the aberrant interaction of newly injected tumor cells with the surrounding stroma, and the draining of injected tumor cells to the secondary lymphoid organs may also lead to an over-interpretation...
of the efficacy of immunotherapeutic intervention (34). In the spontaneous tumor model that was used in this study, the numbers of endogenous T cells specific for the tumor-associated epitope that is targeted are close to background levels at the age tumor development is first seen (Ref. 15; Fig. 2), thereby representing a T cell target Ag for which TIL transfer appears to be precluded. Within this model we show that, whereas vaccination or TCR gene transfer by itself is entirely without effect, the combination of vaccination with TCR gene transfer is highly synergistic. These data directly demonstrate the potential of TCR modified T cells to target tumor types for which classical T cell therapy with autologous tumor-specific T cells has not been possible. As TCRs for Ags such as p53 (35), WT-1 (31), PRAME (N. Rufer, C. Melief, and T. Schumacher, unpublished data), and murine double minute-2 (36) have been isolated, clinical assessment of such therapies can be expected in the coming years.

It seems reasonable to expect that to obtain strong T cell responses in such trials, (at least) three ingredients will be required. First, it should be feasible to generate a sufficient number of autologous gene-modified T cells for infusion. As the current data demonstrate that the infusion of a small number of gene modified T cells (1–5 × 10^7 gene-modified T cells; which equals ~1% of the total CD8^+ T cell compartment) suffices to delay tumor progression, this is unlikely to pose the major problem in future clinical tests. It may, however, be important to ensure that the infused gene-modified T cells have not undergone terminal differentiation (37).

Second, the sole infusion of TCR gene-modified T cells without further treatment is insufficient for the generation of robust tumor-specific T cell responses (Fig. 6). Therefore, a clinically acceptable strategy to promote the expansion of TCR gene-modified T cells is required. Although in the current manuscript, in vivo expansion is achieved by vaccination, host conditioning regimens that have originally been developed for TIL therapy may prove equally or even more effective in this respect (M. de Witte, A. Jorritsma, A. Kaiser, M. van den Boom, M. Dokter, G. Bendle, J. Haenen, and T. Schumacher, unpublished observations). The development of effective strategies to promote in vivo activation of the TCR-modified T cells also appear to be essential to prevent the tolerization of infused TCR-modified T cell populations. In the current study, SV40IV-TCR transduced T cell responses directly after infusion were on average ~1.5–2-fold lower in TRAMP mice than in B6 controls. More importantly, when TCR-modified T cell responses in TRAMP mice were examined months after transfer, reactivity was highly limited. These data are consistent with the notion that the long-term presence of self Ag-reactive T cells in an Ag-bearing host can lead to progressive tolerization (20, 21, 38) and strategies to prevent such tolerization will be valuable.

Third, a collection of (publicly available) high affinity T cell receptors for a series of tumor-associated Ags will need to be created. Biotechnological platforms for TCR isolation may perhaps best be designed by analogy with platforms for recombinant Ab isolation. Specifically, Muromonab-CD3, the first mAb registered for clinical use, was obtained by vaccination of mice and immunogenicity of this fully murine Ab and other Abs of mouse origin has limited their clinical application (39). To avoid the immunogenicity of mouse Abs in clinical use, increasingly sophisticated approaches for the generation of mAbs with a partial or fully human sequence have been developed in subsequent years, eventually resulting in the creation of mouse strains transgenic for the unarranged human Ig loci (40). By analogy to the use of these hIg mouse models for the generation of human Abs against human self-Ags, mouse models that carry the human TCR loci should be of substantial value. Human TCRs obtained from such models may then be used to produce a tumor-reactive T cell repertoire by modification of pre-existing patient derived T cells, or, in the more distant future, such cells may be generated from scratch, through in vitro differentiation of TCR modified T cells from hematopoietic stem cells (41–43).

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Disclosures

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References


