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TITLE: MODULATION OF T CELL TOLERANCE IN A MURINE MODEL FOR
IMMUNOTHERAPY OF PROSTATIC ADENOCARCINOMA

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The goal of this project is to characterize T cell tolerance to prostate tumor antigens and to identify the role of costimulatory receptors in overcoming this tolerance. Identification of these processes will assist in the development of novel therapeutic approaches for treating prostate cancer. We use the TRAMP model, a transgenic mouse line that develops primary prostatic tumors due to expression of the SV40 T antigen (TAg) under the transcriptional control of a prostate-specific promoter. In this final addendum summary, we report that subsequent to adoptive transfer of naïve TAg-specific T cells into TRAMP mice, there is rapid expansion and contraction of the tumor-specific T cells, followed by accumulation of a population of T cells that persist in the prostate as tolerant and suppressive. Co-transfer of TAg-specific CD4⁺ T cells delays the tolerant, suppressive phenotype of prostate-tumor-specific T cells. Transfer of CD4⁺ T cells does not reverse tolerance of previously-tolerized CD8⁺ cells. The suppressive nature of these CD8⁺ T cells was also studied and we demonstrate that suppression is at least in-part mediated by secreted factors. Further, we demonstrate that trafficking of T cells to the TRAMP prostate may be mediated by chemokines. These data demonstrate the critical balance between T cell activation and tolerance and support a mechanism by which tumor growth may induce tolerance and suppressor activity in T cells previously primed to tumor-specific antigens. A greater understanding of how tolerance of these tumor specific T cells can be reversed will certainly lead to more potent anti-tumor immunotherapies.

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Introduction

It is well-appreciated that growing tumors suppress the anti-tumor response by at least 2 mechanisms-generalized immunosuppression and antigen-induced tolerance. The goal of this research project is to test the hypothesis that modulating costimulatory receptors expressed by T cells can reverse tolerance to prostate tumor antigens and elicit a more potent anti-tumor immune response. We use a transgenic mouse model of human prostate cancer, the **TR**ansgenic **A**denocarcinoma of the **M**ouse **P**rostate (TRAMP) model, to study T cell responses to prostatic tumors. In TRAMP mice, primary tumors develop as a consequence of prostate-specific expression of a transforming antigen, the SV40 T antigen (TAg). In this model system, TAg serves as a surrogate tumor antigen. In combination with TRAMP mice, we use two other mouse lines which each bear T cell receptor transgenes that encode either MHC class I-restricted (TcR-I) or class II-restricted (TcR-II) antigen receptors. Our goal is to use these murine lines to understand how T cells develop tolerance to tumor antigens and to test whether modulation of costimulatory receptors is sufficient to overcome tolerance to tumors by understanding these basic immunologic processes.

Body

Task 1: *To determine the Developmental Stage at Which TRAMP Mice Become Tolerant to TAg:*

We initially reported that SV40 TAg expression has a diverse expression pattern in TRAMP mice. mRNA for TAg was detected in the thymus as early as 7.5 weeks of age. This is consistent with another report suggesting central tolerance to TAg in TRAMP mice {Zheng, 2002 #241}. In addition, we observed TAg message in the prostate as early as 1 week of age, which was the earliest time point prostate dissection was possible. Taken together, these findings suggested that TRAMP mice may have both central and peripheral mechanisms to generate tolerance to a tumor antigen. Thus, we next sought to test T cell tolerance using a tumor cell-based vaccination approach.

As previously reported, we attempted to vaccinate TRAMP mice with a syngeneic tumor cell-based vaccine that expressed the full-length SV40 TAg. Despite confirmation of TAg expression by the cell lines, these cells were unable to elicit a TAg-specific T cell response in wild-type mice, nor were they able to stimulate TcR-I cell *in vitro*. As a result, we were unable to use this cell line as a vaccine to test TRAMP tolerance to TAg. Similarly, the TcR-I peptide emulsified in adjuvant was only a weak stimulator of T cell responses in wild-type mice. Therefore, we focused our efforts on the adoptive transfer studies which are presented in Aim 3.

Task 2: *To test the hypothesis that blockade of CTLA-4/B7 interactions, alone, or in combination with modulation of the costimulatory receptors CD40 and 4-1BB, can reverse tolerance to TAg in TRAMP mice*

As described in the 2003 Annual Report, we did not pursue this Specific Aim due to the difficulties in generating a TAg-specific vaccine, as described in Task 1, above. Our effort focused on the adoptive transfer model system, which has generated significant and highly relevant data.

Task 3: *To use an adoptive transfer system where transgenic T cells that recognize MHC class I- and class II-restricted TAg epitopes can be monitored to test the hypothesis that a developing prostatic tumor can tolerize naïve TAA-specific T cells.*

Many studies suggest that as a tumor develops, T cell tolerance to TAA's ensues. Most of these studies have employed transplantable tumor lines that express xenogeneic antigens that are thus

highly stimulatory to the immune system. The TRAMP model presents a novel model where primary tumors develop under the developmentally regulated expression of a tissue-restricted promoter. We proposed to study T cell tolerance using the TcR-I and TcR-II transgenic lines which bear transgenes encoding TcR genes that recognize MHC class I- and class II-restricted epitopes of TAg, respectively.

The TcR-I mouse strain was bred to homozygosity on the C3H background. Lymph node cells (LNCs) from these mice were used as donor cells for transfer in TRAMP x C3H (TRAMP/F₁) mice. Similar transfers were performed using wild-type C57BL/6 x C3H (WT/F₁) as recipients or using WT C3H cells as donor cells. Donor LNC were labeled with CFSE, a fluorescent dye that distributes evenly among daughter cells as the cells divide and therefore a linear reduction of fluorescence is observed at each mitotic division.

In the previous final report, we presented data that characterized TcR-I responses in TRAMP mice. TcR-I cells undergo an initial expansion followed by an apoptotic contraction that results in deletion from the peripheral lymphoid tissues and trafficking of a fraction of cells to the prostate. These tumor-infiltrating cells persist as tolerant in the TRAMP prostate. We further demonstrated that a dendritic cell (DC) vaccine can prime TcR-I cells in TRAMP mice, prevent tolerization for up to 3 weeks, and reduce tumor burden, as indicated by prostate size. However, over time, persistence of TAg expression tolerizes TcR-I cells and tumor growth is restored. These data demonstrate that despite initial successful priming, TcR-I cells become tolerized due to progressive tumor growth. These findings support our hypothesis and have critical implications when considering tumor vaccines. These findings were published in the *Journal of Immunology*.

CD4⁺ T Cell Responses to TRAMP Tumors

In the previous report, we described our initial studies of TcR-II cell transfer into TRAMP mice. We have extended those studies to examine the full kinetics of TcR-II cell responsiveness after transfer. As mentioned previously, TcR-II cell trafficking is similar to TcR-I trafficking, although somewhat delayed with respect to appearance in the prostate. When they first appear in the prostate 3-5 days after transfer, TcR-II cells exhibit diminished but detectable responses. However, by 10 days after transfer, T cells are completely unresponsive and do not proliferate or produce interferon- γ (IFN- γ) or interleukin-2 (IL-2). This persists up to 30 days after transfer.

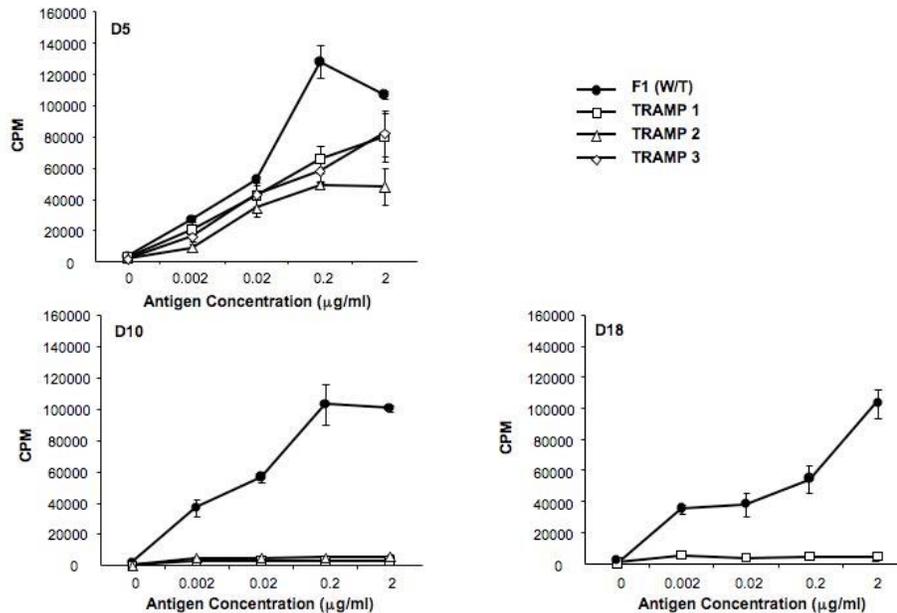


Figure 1: TRAMP or Wild-type (F1(W/T)) mice were transferred with 5×10^6 TcR-II cells. Mice were euthanized at the indicated time points and TcR-II cells enriched using anti-Thy1.1-conjugated magnetic beads. Cells were placed into culture with the indicated antigen concentration and splenocytes and proliferation measured following pulse with ^3H -thymidine.

Co-transfer of TcR-II Cells Delays Induction of TcR-I Cell Tolerance

Given the transient activation of TcR-II cells, we tested whether co-transfer of TcR-I cells with TcR-II cells would prevent TcR-I cell tolerance. In our previous report, we presented preliminary data that like a dendritic cell vaccine, co-transfer of TcR-II cells with TcR-I cells could delay TcR-I tolerization. Mice were pre-transferred with TcR-II cells and 18-24 hours later, transferred with CFSE-labeled, Thy1.1⁺ TcR-I cells. At various time points after transfer, TcR-I cells were purified from prostatic tissue using anti-Thy1.1-conjugated magnetic beads and subsequently used in functional assays to test antigen responsiveness. As demonstrated in Figure 2, pre-transfer of TcR-II cells could delay the onset of TcR-I tolerization in TRAMP mice. These data suggest that the transient activation of tumor-specific CD4⁺ cells can support the expansion and activation of CD8⁺ T cells. However, over time, the immunosuppressive environment of the tumor persists and the CD8⁺ T cells become tolerized. Thus, our on-going studies are aimed at testing whether continued transfer of naïve CD4⁺ T cells can sustain CD8⁺ T cell responsiveness.

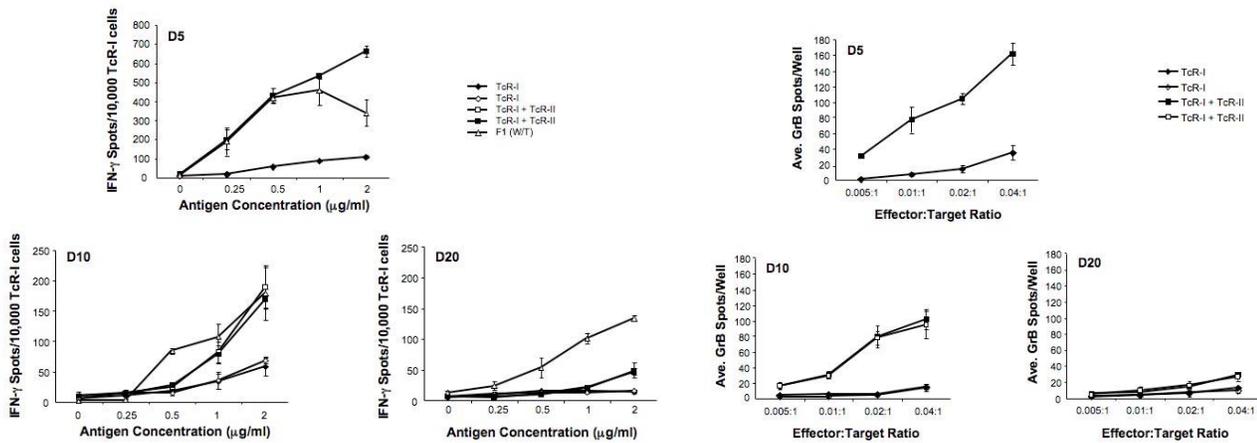


Figure 2: Mice were transferred with TcR-I cells alone or pre-transferred with TcR-II cells (18-24 hours prior). At the indicated times after transfer, prostatic tissues were harvested and TcR-I cells isolated using anti-Thy1.1-conjugated magnetic beads. Enriched TcR-I cells were placed into plates pre-coated with anti-IFN-g or anti-GranzymeB and splenocytes and antigen added. Plates were developed for ELISPOT using matched antibody pairs.

TRAMP Tumors Induce Suppressor Activity in CD8⁺ T Cells

In the previous report, we described our on-going studies characterizing the suppressive activity of prostate-infiltrating T cells. We had observed that TcR-I cells isolated from the TRAMP prostate not only were tolerant of their cognate antigen, but also were capable of suppressing other CD8 T cells from proliferating. In the intervening time, we have done extensive studies to confirm that this activity is not due to any contamination of the enriched cells. In addition, we have extended these findings as follows:

We further studied the ability of TRAMP tumor infiltrating TcR-I cells to suppress both CD4 and CD8 T cell proliferation. As shown in figure 3, TcR-I cells were able to suppress CD8 T cells specific for a melanoma antigen, TRP-2, as well as ovalbumin-specific CD4 T cells.

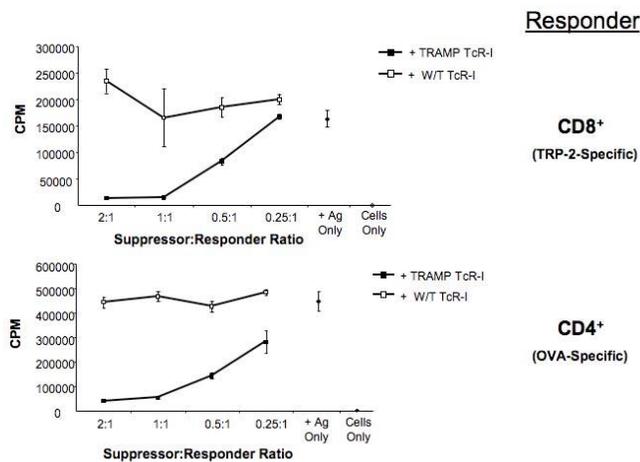


Figure 3: TcR-I cells were purified as described in Figure 1 and added to a proliferation assay using either CD8⁺ T cells or CD4⁺ T cells as responder cells. Proliferation was assessed using ³H-thymidine incorporation.

To determine whether suppression is mediated by cell-cell contact or soluble mediators, we prepared supernatants from the purified TcR-I cells and tested them in a similar proliferation assay. As

demonstrated in Figure 4, even at high dilutions, the supernatants from freshly-isolated TcR-I cells were capable of suppressing proliferation of both CD8⁺ and CD4⁺ (not shown) T cells.

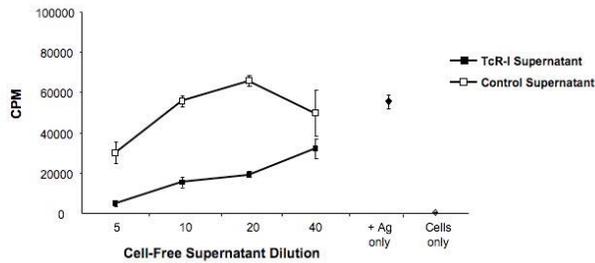


Figure 4: TcR-I cells were purified using anti-Thy1.1-conjugated magnetic beads and placed in culture for 48 hours. Supernatants were collected, frozen and used in subsequent proliferation assays. Briefly, naïve TRP-2-reactive T cells were stimulated with antigen in the presence of TcR-I supernatants using the indicated dilutions. Proliferation was assessed using ³H-thymidine incorporation.

Expression of Chemokines by TRAMP Prostate Tumors

To study the mechanism by which T cells preferentially traffic to the TRAMP prostate, we used a PCR-based approach (Superarray, Inc). This platform allowed us to quantitate the levels of expression of 85 cytokines and chemokines in the TRAMP prostate and compare the levels of expression with prostate tissues from wild-type mice. As shown below, there was increased expression of several T cell chemokines in the TRAMP prostate.

Chemokine	Fold-Increase	P-value
Ccl 5 (RANTES)	6.2	<0.0001
Cxcl 10 (IP-10)	5.6	0.04
Cxcl 11 (I-TAC)	3.6	0.002
Ccl 22 (MDC)	3.2	0.04
foxP3	3.9	0.02

Our on-going studies are now testing chemokine receptor expression on TcR-I cells that infiltrate TRAMP prostates. Once we determine the repertoire of receptor expression, we will attempt to block chemokine signaling by either treating with chemokine receptor-specific antibodies or use si-RNA to reduce receptor expression.

Key Research Accomplishments

Establishment of adoptive transfer model

Characterization of TcR-I cells transferred into TRAMP mice

Identification of DC vaccine as effective in preventing deletion of TcR-I cells and promoting survival and expansion of TcR-I cells in prostate

Identification of TcR-II cells as effective in preventing initial deletion and preventing tolerization of TcR-I cells in co-transfer studies

Identification and partial characterization of suppressor activity of tolerant TcR-I cells

Identification of chemokine expression by TRAMP prostate tumors

Reportable Outcomes:

One manuscript published in *The Journal of Immunology*.

Conclusions:

Our long-term goal is to understand the role of costimulatory receptors in regulating T cell tolerance to tumor antigens. Our early data suggest that TRAMP mice may exhibit both central and peripheral tolerance to TAg, a surrogate tumor antigen. We have focused our research on using the TRAMP mouse as a recipient for TAg-specific T cells.

Our findings suggest that prostate-specific T cells undergo an initial proliferative response after antigen encounter. This is followed by deletion from the peripheral lymphoid organs and the prostate, the site of antigen expression. However, sensitization with an antigen-pulsed DC vaccine, or provision of a naïve CD4⁺, tumor-specific T cell, prevents deletion of prostate-specific T cells and delays tolerance induction. The T cells that persist on the prostate exhibit a potent suppressive activity that may be a critical factor to overcome when attempting to elicit anti-tumor immune responses.

Our on-going studies are characterizing both the deletional tolerance process as well as the mechanism by which the DC vaccine rescues T cells. In addition, we are studying the mechanisms by which these tolerant, CD8⁺ cells exert their suppressor function. Finally, we are studying the mechanisms by which T cells traffic to the prostate, which may be critical for enhancing cancer vaccines.

References:

None

