Recombinant Adenovirus Vaccine Encoding a Chimeric T-Cell Antigen Receptor Induces Protective Immunity against a T-Cell Lymphoma

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ABSTRACT

Vaccination using recombinant tumor-derived T-cell antigen receptor (TCR) protein induces a protective, idiotype-specific immune response against a murine T-cell tumor. However, the technically demanding task of producing patient-specific, recombinant TCR protein restricts the translation of TCR vaccines for clinical use. We report here the development of an effective recombinant TCR adenovirus vaccine. Individual adenosines were constructed to encode a chimeric TCR derived from either tumor V\(\alpha\) or V\(\beta\) regions fused to xenogeneic human constant regions. Coinjection of the chimeric \(\alpha\)- and the \(\beta\)-TCR adenosines protected mice against tumors. The level of protection was comparable to that achieved by an optimized regimen of recombinant TCR protein vaccines. Tumor immunity induced by TCR adenoviruses required the xenogeneic constant regions and was mediated by CD8\(^+\) T cells. Independent vaccines consisting of adenoviruses expressing either chimeric \(\alpha\)- or \(\beta\)-TCR chain also stimulated a protective immune response. Immunization with TCR adenosine may offer a new efficacious, protein-free vaccination approach for the treatment of T-cell malignancies.

INTRODUCTION

T-cell malignancies (lymphomas and leukemias) represent a diverse class of cancers that remain largely incurable and fatal in the majority of patients despite modern chemotherapy, radiation therapy, and bone marrow transplantation (1–3). A new alternative approach using immunotherapy that targets specific markers expressed on the surface of these malignant T cells is being developed. The variable regions encoded on the Ag\(^+\) receptors of normal B and T cells arise from recombination events between the V, (D), and J gene segments of Ig and TCR, respectively. This process gives rise to antigenic determinants (Ids) that are unique to each Ag receptor. The Ids expressed on B- and T-cell lymphomas are therefore ideal candidates for use as tumor-specific targets for cancer immunotherapy. Our group previously tested the use of Id vaccines for the immunotherapy against T-cell lymphomas and demonstrated that recombinant tumor-derived TCR proteins could be formulated into an effective vaccine that stimulated specific protection against tumor in our murine T-cell lymphoma model (4). Recombinant TCR protein vaccines induced both humoral and cellular anti-Id immune responses. It was demonstrated that antitumor immunity was primarily mediated by CD8\(^+\) T cells (5). This is in contrast to findings in several murine B-cell lymphoma models where protection against tumor was primarily mediated by an anti-Id Ab response (6–8). Although the recombinant TCR protein vaccines proved efficacious in our murine T-cell tumor model, it required the production of a soluble TCR protein that was difficult to obtain. The feasibility of translating TCR vaccines into clinical practice is presently limited because of the laborious task of producing custom-made TCR protein for individual patients in sufficient quantities.

In this study, we developed an adenovirus vaccine approach for the treatment of a murine T-cell lymphoma. Recombinant adenovirus vaccine has several advantages over our TCR protein vaccine strategy: (a) The inherent immunogenicity of adenoviral vectors had been well documented to induce immune responses against the product encoded by the transgene (9, 10). Antiviral immunity may provide an adjuvant effect in the induction of immune response against tumor-specific Ags in cancer immunotherapy. (b) Adenoviral vectors deliver TCR into the endogenous Ags processing pathway that directs peptide processing and presentation onto class I MHC. This should be more potent in the activation of CD8\(^+\) T cells than soluble protein vaccines because soluble Ags are mostly taken up by the exogenous Ag processing pathway and presented on class II MHC. (c) The recent development of a simplified system for generating recombinant adenoviruses allows for the rapid production of patient-specific adenoviral vectors (11, 12), eliminates the technically challenging hurdle of producing custom-made TCR protein, and should potentially make Id TCR vaccines clinically feasible.

We report here the construction of recombinant adenovirus vaccines coding for the TCR derived from the murine tumor C6VL. Viral vector coding for the native TCR was constructed. In addition, individual adenosines were constructed to encode a chimeric TCR derived from either tumor V\(\alpha\) or V\(\beta\) regions fused to xenogeneic human constant regions. Mice coinjected with adenoviruses coding for the \(\alpha\) and \(\beta\) chimeric TCR exhibited strong anti-Id immune responses and were protected against tumor. In contrast, mice vaccinated with adenovirus coding for the native TCR did not show any detectable immune response and were not protected against tumor. The protective effect of chimeric TCR adenovirus vaccine was dependent on CD8\(^+\) T cells. The contribution of \(\alpha\)- and \(\beta\)-TCR chains to tumor protection was investigated. Adenovirus encoding independent TCR \(\alpha\) and \(\beta\) chains with xenogeneic constant regions were each effective in conferring tumor protection. This new TCR vaccination strategy may offer a new approach to the treatment of T-cell malignancies.

MATERIALS AND METHODS

Mice and Cell Lines

Female C57BL/6 mice (H-2\(^b\); aged 6–8 weeks) were purchased from Charles River Laboratories (Wilmington, MA). C6VL (H-2\(^b\)) is a murine tumor cell line (13). Rat hybridomas 2.43 (rat IgG2b antimouse CD8.2) was obtained from American Type Culture Collection (Rockville, MD). Rat hybridomas SFR8-B6 (rat IgG2b antihuman HLA Bw6) was kindly provided by J. Parnes (Stanford, CA). 293A is a subclone of 293, the human embryonic kidney cell line that was transformed by sheared human adenovirus type 5 DNA (Quantum Biotech, Quebec, Canada). B15183 is a recombination-proficient strain of bacteria (kindly provided by M. Mehtali, Transgène SA, France).
Genetic Constructs

Genes coding for PI-linked C6VL TCR α and β sequences were previously constructed for protein expression in the psRsnD5 vector (4), with the transmembrane domain of TCR replaced with sequences derived from the 3' region of the DAF that encodes for a PI linkage domain (14). The bicistronic αβ-TCR/MoCR was assembled in the vector ptCACE 5.3 as follows: DNA encoding for PI-linked C6VL α-TCR was excised from the psRsnD5 vector and transferred into ptCACE 5.3. The sequence for IRES (15) was inserted downstream of the α chain (plasmid encoding IRES was kindly provided by G. Nolan, Stanford University). DNA encoding for PI-linked C6VL β-TCR was cloned downstream of IRES.

The αβ-TCR containing human TCR constant regions (α-TCR/HuCr/DAF and β-TCR/HuCr/DAF) were constructed as follows: C6VL, Vα and Vβ were PCR-amplified from psRsnD5 vectors and cloned into separate ptCACE 5.3 vectors. Human TCR Cα and Cβ (without transmembrane regions) were PCR-amplified from human peripheral blood lymphocyte cDNA and cloned inframe downstream of the respective C6VL Vα and Vβ genes. Sequences for IRES domain of the DAF gene was PCR-amplified from the psRsnD5 vector and inserted inframe downstream of the human TCR α and β genes.

The assembled αβ-TCR/MoCr/DAF, α-TCR/HuCr/DAF, and β-TCR/HuCr/DAF were cloned from ptCACE 5.3 into the adeno transfer vector pXCI-CMV/V/α (pXCI). pXCI is a mammalian expression vector that contains adenoviral sequences downstream of a 0–452 bp, and 3328–5789 bp of the Ad 5 genome flanking the multiple cloning site (kindly provided by L. Verma, Salk Institute, San Diego). The adenovirus-derived sequences facilitate the homologous recombination of TCR into the E1 locus of the adenovirus type 5 genome encoded on the plasmid ptG3652 (kindly provided by M. Mehtali, Transgène SA, France; Ref. 11).

Generation of Recombinant Adenovirus

Plasmid Recombination. Recombination of pXCI and ptTG3652 plasmids in bacteria were performed as previously reported (11, 12) with slight modifications. Linearized pXCI vectors containing various forms of C6VL TCR were cotransfected with C6VL-linearized, ptTG3652 into recombination-proficient BJ5183 cells. Recombination was determined by restriction digest mapping of plasmid DNA from bacterial colonies. The recombined plasmid was retransformed into XLI1 Blue cells for large-scale plasmid purification.

Transfection of 293 Cells with Recombinant Adenoviral DNA Plasmid. 293A cells were transfected with 1 μg PacI-linearized, recombined TG3652 containing various TCR genes using Lipofectamine Plus (Life Technologies, Gaithersburg, MD). One day after transfection, transfected cells were immobilized with media containing 0.5% agarose and monitored for viral plaque formation. Viral plaques were isolated and used to generate primary and amplified viral stocks. Correct viral clones were confirmed by PCR analysis.

Adenovirus Purification. Purification of adenovirus was done according to published reports with slight modification (16). 293A cells were infected with amplified viral stock diluted into sterile PBS. When cytopathic effect was apparent, infected cells were harvested, resuspended in 10 mM Tris (pH 8)-1 mM EDTA, and lysed by three freeze/thaw cycles. Viral lysate was mixed with amplified viral stock diluted into sterile PBS. When cytopathic effect was developed, starting 1 week after the third mAb injection, serum samples were collected 10 days after each immunization.

Adenovirus Vaccination. Groups of 10 C57BL/6 mice were vaccinated i.m. once with 10^9 pfu purified adenovirus encoding for αβ-TCR/MoCr/DAF, and β-TCR/HuCr/DAF were cloned from ptCACE 5.3 into the adeno transfer vector pXCI-CMV/V/α (pXCI). pXCI is a mammalian expression vector that contains adenoviral sequences downstream of a 0–452 bp, and 3328–5789 bp of the Ad 5 genome flanking the multiple cloning site (kindly provided by L. Verma, Salk Institute, San Diego). The adenovirus-derived sequences facilitate the homologous recombination of TCR into the E1 locus of the adenovirus type 5 genome encoded on the plasmid ptG3652 (kindly provided by M. Mehtali, Transgène SA, France; Ref. 11).

Protein Vaccination. Recombinant C6VL αβ-TCR protein was obtained as previously described (4). Purified C6VL αβ-TCR protein was chemically conjugated to KLH (Calbiochem, San Diego, CA) at a 1:1 ratio (w/w) using glutaraldehyde as previously described (4). Mice were immunized s.c. with TCR-KLH conjugates containing 35 μg (500 pmol) of TCR mixed with 10 μg of QS-21 adjuvant (kindly provided by Aquila Biopharmaceuticals, Framingham, MA; Ref. 17). The irrelevant protein vaccine control consisted of IgD protein derived from the murine B-cell lymphoma 38C13 (18), similarly conjugated to KLH and given in equal molar amount in QS-21. Immunizations were given three times at 2-week intervals. Serum samples were collected 10 days after each immunization.

Anti-C6VL αβ-TCR ELISA Assay

ELISA assays were performed as previously described (4). Briefly, 96-well Maxisorb plates were coated with anti-Cβ mAb H57–597. Purified C6VL αβ-TCR was bound to H57–597-coated plates. Mouse immune serum was titered over 8 wells in 2-fold dilutions. A standard curve was generated by titering mAb 124–40, a mouse IgG anti-C6VL TCR clonotype encoded on the α chain variable region. Bound mouse Abs were detected using a peroxidase-conjugated donkey antimouse Ig Ab (Jackson ImmunoResearch, West Grove, PA). Color reaction developed using an azinobis(3-ethyl)benzthiazoline sulfonyl acid substrate was measured using a Vmax microplate reader at 405–450-mm absorbance (Molecular Devices, Menlo Park, CA).

In Vivo Depletion of CD8+ T Cells

C57Bl/6 mice were depleted of CD8+ T cells using the mAb 2.43. Control mice were injected with an irrelevant isotype-matched mAb SFR8-B6. Mice were injected i.p. with 250 μg of mAb in 500 μl of PBS on days 6, 5, and 4 before tumor challenge. Three weekly injections were given after tumor challenge to maintain the depletion, starting 1 week after the third mAb injection. The extent of CD8+ T-cell depletion in peripheral blood was analyzed by flow cytometry 1 day before tumor challenge and 3 days after the last weekly mAb treatment using a nonblocking anti-CD8 mAb (PharMingen).

Tumor Challenge

Two weeks after the third protein vaccination, or 2 weeks after a single adenovirus vaccination, mice were challenged with a lethal dose of C6VL cells. A frozen aliquot of C6VL cells was thawed and was grown in RPMI 1640 containing 10% FCS and 50 μM of 2-mercaptoethanol for 2 days. The tumor cells were collected, washed three times in HBSS, and diluted to 2 × 10^6 cells/ml. Mice were injected i.p. with 1000 C6VL tumor cells in 500 μl of HBSS. Survival of mice challenged with tumor was monitored for at least 60 days after tumor injection. Survival curves were generated using the graphics...
RESULTS

Generation of Recombinant Adenovirus Vaccines for C6VL TCR. We constructed a replication-defective recombinant adenovirus coding for the TCR of the murine T-cell lymphoma C6VL. Genes coding for the TCR α and β chains were joined by an IRES (15) to allow for protein translation of both α and β chains from a single mRNA transcript. The TCR transmembrane regions were replaced with a PI linkage domain that directs cell surface expression. Recombinant adenovirus coding for native, PI-linked C6VL TCR was assembled according to the simplified method reported by Chartier et al. (11), where homologous recombination of TCR into the E1/E3-deleted adenoviral DNA into the 293A cells reliably produced infectious, replication-defective adenovirus particles with the correct transgene. Homogeneous viruses can be obtained from this procedure without multiple rounds of plaque purification. HeLa cells infected with adenovirus coding for C6VL TCR (αβ-TCR/MoCR/Ad) expressed the αβ-TCR on the cell surface (Fig. 1A). TCR expression level was dose-dependent and correlated with the MOI used during adenovirus infection (data not shown).

To increase the immunogenicity of the native TCR, a second set of TCR adenoviruses were made in which the murine TCR constant regions were replaced with those derived from the human TCR α or β constant regions. A separate adenovirus was made for the chimeric murine-human α-chain TCR and β-chain TCR (α-TCR/HuCR/Ad and β-TCR/HuCR/Ad, respectively) to allow for ease of gene construction and the ability to analyze each TCR chain independently. HeLa cells coinfectected with both α-TCR/HuCR/Ad and β-TCR/HuCR/Ad expressed both chimeric α and β TCR chains on the cell surface (Fig. 1B). The chimeric TCR retained its secondary structure as recognized by the clonotypic anti-C6VL TCR mAb 124–40 (data not shown). HeLa cells infected with either the α-TCR/HuCR/Ad or the β-TCR/HuCR/Ad also expressed surface TCR (Fig. 1C). The difference in mean fluorescence levels observed between α-TCR/HuCR/Ad-infected and β-TCR/HuCR/Ad-infected cells could not be directly compared because of the use of different mAbs in flow cytometry analysis.

Induction of Humoral Immune Responses in TCR Adenovirus-vaccinated Mice. The immunogenicity of TCR adenovirus vaccines was evaluated in mice vaccinated with a single i.m. injection of adenovirus coding for (a) native C6VL TCR (αβ-TCR/MoCR/Ad), (b) chimeric murine-human TCR consisting of a mixture of α-TCR/HuCR/Ad and β-TCR/HuCR/Ad (α+β-TCR/HuCR/Ad), or (c) separate chimeric TCR chains (α-TCR/HuCR/Ad or β-TCR/HuCR/Ad). Control mice were vaccinated with Luc/Ad. Serum samples were collected from vaccinated mice before and after vaccination and analyzed for anti-C6VL TCR Ab by ELISA using purified PI-linked TCR protein as a target on microtiter plates. Despite the inherent immunogenicity of adenovirus as a carrier vehicle, mice vaccinated with αβ-TCR/MoCR/Ad did not make any detectable humoral immune response against C6VL TCR (Fig. 2A). In contrast, mice vaccinated with α+β-TCR/HuCR/Ad developed strong levels of anti-C6VL TCR Abs. The humoral response induced was specific for the C6VL TCR because hyperimmune serum from mice vaccinated with α+β-TCR/HuCR/Ad did not react with two different human T-cell lines (data not shown). Anti-TCR Abs were detected within 7 days after vaccination, with Ab titers peaking at 1–2 weeks and gradually decreasing over a month. Mice vaccinated with β-TCR/HuCR/Ad alone had comparable Ab titers to those given α+β-TCR/HuCR/Ad, with average Ab titers of 10 μg/ml and 14 μg/ml, respectively (Fig. 2B). α-TCR/HuCR/Ad-vaccinated mice did not make detectable levels of anti-TCR Ab.

Induction of Antitumor Immunity in TCR Adenovirus-vaccinated Mice. The antitumor effect of TCR adenovirus vaccines was tested by challenging adenovirus-vaccinated mice with a lethal dose...
of C6VL cells and following them for survival (Fig. 3). In accordance with the lack of an Ab response, mice vaccinated with αβ-TCR/MoCR/Ad did not acquire protective immunity and died at a similar rate as mice vaccinated with control adenovirus. In contrast, protective antitumor immunity was induced in mice vaccinated with the chimeric αβ TCR adenoviruses. Length of survival of αβ-TCR/HuCR/Ad-vaccinated mice was significantly better compared to the survival of mice vaccinated with αβ-TCR/MoCR/Ad or Luc/Ad (P = 0.0001 and 0.0008, respectively). Tumor protection by the αβ-TCR/ HuCR/Ad vaccine was repeated in four independent experiments. The protective effect of the TCR adenovirus vaccine required the presence of the xenogeneic human TCR constant regions. However, vaccination did not result in a global deletion of T cells because the percentages of CD4+ and CD8+ T-cell populations in draining lymph nodes (Table 1) and peripheral blood lymphocytes (data not shown) were not affected in TCR adenovirus-vaccinated mice. Furthermore, in TCR adenovirus-vaccinated mice, the percentage of normal T cells that used similar variable regions as C6VL TCR (Vα3.1 and Vβ6) were not altered (Table 1).

**Comparison of TCR Protein and Adenovirus Vaccines in the Induction of Antitumor Immunity.** To evaluate whether the level of tumor protection induced by the adenovirus vaccine was comparable to the previously developed protein vaccines, a direct comparison was set up using the optimal vaccination regimen. Groups of 10 mice were vaccinated either once i.m. with αβ-TCR/HuCR/Ad or three times biweekly with TCR-KLH protein given s.c. in QS-21 adjuvant. Two weeks after the single adenovirus vaccination and the last protein vaccine, mice were challenged with C6VL tumor and followed for survival. Mice vaccinated with TCR-KLH protein or αβ-TCR/HuCR/Ad were significantly protected against tumor as compared to mice vaccinated with αβ-TCR/MoCR/Ad (P = 0.0001 and 0.03, respectively; Fig. 4). The levels of tumor protection achieved using these two forms of vaccinations were equivalent (P = 0.31). Mice given only a single TCR protein vaccination according to the schedule used for virus vaccination were not protected and died at a similar rate as control mice (data not shown). The level of tumor protection by TCR vaccines varied in different experiments, and statistical significance was always reached (P < 0.05) when compared to negative control groups.

**Role of CD8+ T Cells and Abs in Tumor Protection in TCR Adenovirus-vaccinated Mice.** We previously demonstrated that in mice vaccinated with TCR protein vaccines, CD8+ T cells were responsible for mediating protection against tumor challenge (5). The importance of effector CD8+ T cells in αβ-TCR/HuCR/Ad-vacci-

![Fig. 2. Induction of anti-C6VL TCR Abs in adenovirus-vaccinated mice. A, groups of five C57Bl/6 mice were vaccinated i.m. with 10⁸ pfu of control/Ad (○), αβ-TCR/MoCR/Ad (△), or αβ-TCR/HuCR/Ad (○). Serum samples were collected weekly and analyzed by ELISA for anti-C6VL TCR Abs. Average Ab titers were represented in each group ± SE.](Image 53x264 to 287x427)

![Fig. 3. Survival of mice vaccinated with TCR adenovirus vaccines and challenged with C6VL tumor. Groups of 10 C57Bl/6 mice were vaccinated i.m. with 10⁸ pfu of control/Ad (○), αβ-TCR/HuCR/Ad (△), or αβ-TCR/MoCR/Ad (○). Two weeks after vaccination, mice were injected i.p. with 1000 C6VL cells and monitored for survival.](Image 318x104 to 550x268)

![Fig. 4. Survival of mice vaccinated with TCR protein vaccines or adenovirus vaccines and challenged with C6VL tumor. Groups of 10 C57Bl/6 mice were vaccinated with a single i.m. injection with 10⁸ pfu of αβ-TCR/HuCR/Ad (○), αβ-TCR/MoCR/Ad (△), or three biweekly s.c. injections of TCR-KLH protein conjugate in QS-21 (○). Two weeks after one adenovirus vaccination or after the third protein vaccination, mice were injected i.p. with 1000 C6VL cells and monitored for survival.](Image 63x509 to 276x741)

**Table 1. Lymph node T-cell percentages in TCR adenovirus-vaccinated mice**

<table>
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<tr>
<th>Vaccine group</th>
<th>CD4 (%)</th>
<th>CD8 (%)</th>
<th>CD3+ Vα3.1 (%)</th>
<th>CD3+ Vβ6 (%)</th>
</tr>
</thead>
<tbody>
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<td>23.2</td>
<td>4.3</td>
<td>5.1</td>
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<td>22.9</td>
<td>3.9</td>
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<tr>
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<td>30.6</td>
<td>22.1</td>
<td>3.1</td>
<td>5.1</td>
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<tr>
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<td>22.0</td>
<td>2.9</td>
<td>5.6</td>
</tr>
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<td>18.4</td>
<td>3.0</td>
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</table>

* Mice were vaccinated with 10⁸ pfu of various adenoviruses. Two weeks after vaccination, mice were sacrificed, and draining lymph nodes were collected for analysis by flow cytometry.

![Table 1](Image 108x119 to 310x325)
Vaccination, mice were injected i.p. with 1000 C6VL cells and monitored for survival. From each other (respectively), the three survival curves were not statistically different. Although the group vaccinated with HuCR/Ad vaccination (Fig. 5). Treatment of nated mice was tested by depleting CD8 cells was not tested in this experiment because anti-CD4 Abs would bind to the CD4+ C6VL tumor cells in addition to normal CD4+ T cells.

Effectiveness of Adenovirus Vaccines Coding for Independent α- or β-TCR. The construction of C6VL TCR α- and β-TCR/HuCR on separate recombinant adenoviruses allowed us to evaluate the relative contribution of each TCR chain to tumor protection. The efficacy of single-chain TCR adenovirus vaccines in inducing antitumor immunity was compared to mice vaccinated with α+β-TCR/HuCR/Ad (Fig. 6). Mice vaccinated with α- or β-TCR/HuCR/Ad alone had an intermediate level of protection compared to mice vaccinated with α+β-TCR/HuCR/Ad, and all three groups had significant protection against C6VL as compared to controls (P < 0.05). Although the group vaccinated with α+β-TCR/HuCR/Ad had more long-term survivors of C6VL tumor compared to the groups vaccinated with either α- or β-TCR/HuCR/Ad alone (50% versus 20%, respectively), the three survival curves were not statistically different from each other (αβ versus α, P = 0.08; αβ versus β, P = 0.17, α1 versus β1, P = 0.46). Interestingly, in two other independent experiments, the percentage of long-term survivors with α+β-TCR/HuCR/Ad vaccination was consistently higher than that with α- or with β-TCR/HuCR/Ad given alone (data not shown).

**DISCUSSION**

The concept of Id vaccination has been well established for the immunotherapy of B-cell lymphomas in murine tumor models (18, 19), and promising results have been reported in ongoing human clinical trials (20, 21). Tumor-derived TCR is an attractive natural tumor-specific Ag that can be used as a target for cancer immunotherapy because TCR Id shares many similarities with Ig Id. Our group has previously shown that active immunotherapy against a T-cell lymphoma could be accomplished using soluble TCR protein formulated into a vaccine (4). TCR protein vaccines were able to induce both humoral and cellular immunity. The induction of antitumor TCR Abs was used as a measure for vaccine immunogenicity; however, the levels of Abs induced did not correlate with tumor protection in the vaccinated mice. CD8+ T cells were shown to play a critical role in mediating tumor protection (5). Protein vaccines, however, are not the optimal vaccination strategy for activating TCR Id-specific CD8+ T cells. Furthermore, producing sufficient quantities of soluble TCR protein is time consuming, labor-intensive, and limits the subsequent optimization of patient-specific TCR vaccines.

Recombinant adenoviruses can infect a broad range of cells, including muscle and dendritic cells (22, 23). Products encoded by recombinant adenoviruses are targeted to the endogenous protein processing pathway and can lead to the induction of a CD8+ T-cell response (24). The development of recombinant adenoviruses as vectors for cancer vaccines has been reported in various animal models (25, 26) and shown to be safe in Phase I clinical studies (27, 28). Therefore, in this study, we tested the ability of recombinant TCR adenoviruses in inducing protective immunity against the murine T-cell tumor C6VL. Replication-defective adenoviruses were constructed using a simplified bacterial recombination system that allowed for rapid screening and production of recombinant viral vectors (11). Adenovirus coding for a PI-linked TCR derived from C6VL, as well as chimeric TCRs containing C6VL TCR V regions fused to human C regions were constructed. Recombinant TCRs were detected on the surface of viral-infected cells (Fig. 1). Both native and chimeric TCRs were expressed at comparable levels, as determined by a clonotypic mAb that was specific against C6VL TCR (data not shown).

Mice vaccinated with adenovirus coding for the native TCR did not generate any measurable anti-TCR humoral immune response and were not protected against tumor challenge (Fig. 3). One possible reason for the lack of protection was the nonimmunogenic nature of the native TCR. This finding is in agreement with results of our protein vaccines, where TCR protein alone did not induce antitumor immunity (4). It was necessary to conjugate TCR protein to an immunogenic carrier protein and coinject with an adjuvant to induce antitumor immunity. In our present study, the administration of native TCR in the context of an immunogenic adenoviral vector was not sufficient to elicit the induction of an anti-TCR immune response. Recent studies of recombinant adenoviral vectors suggest that antigene immune responses are primarily determined by the inherent immunogenicity of the transgene product, and the role of antiadenovirus immunity may be overestimated (29, 30).
The requirement of additional components to improve the immunogenicity of a weak Ag in genetic vaccines has been reported. This was achieved, for example, by fusing Ag to the cytokine granulocyte colony-stimulating factor (31). This was thought to enhance Ag presentation by dendritic cells. Ag immunogenicity was also improved by fusing Ag to tetanus toxoid fragment C protein, attributable to cross-priming and the recruitment of carrier-specific T-cell help (32, 33). We have previously shown that although the induction of anti-TCR Ab response cannot be correlated to tumor protection, it nonetheless served as a marker for vaccine immunogenicity (4, 5). In this study, native TCR adenovirus was made immunogenic by replacing the syngeneic TCR constant region with a xenogenic constant region from human. Mice vaccinated with adenovirus coding for the chimeric TCR induced anti-C6VL TCR Abs (Fig. 2). Ab responses were detectable within 1 week after vaccination, indicating the effectiveness of the adenovirus in transgene delivery. The level of Ab response only persisted for 1 month, suggesting cells infected with the chimeric TCR adenovirus were cleared because of anti-TCR and antiviral immune responses. The induction of anti-TCR immune responses significantly protected vaccinated mice from tumor (Fig. 3). The contribution of the human constant region was critical in an effective TCR adenovirus vaccine, with the human constant region likely acting as an immunogenic carrier protein. The antitumor immunity induced using the chimeric TCR adenoviruses was not directed against TCR constant regions because no change in the percentages of T cells was observed in chimeric TCR adenovirus-vaccinated mice (Table 1). In addition, anti-TCR immune response was Id-specific; the percentages of T cells that used the same V region as C6VL TCR were unchanged in mice vaccinated with the chimeric TCR adenovirus (Table 1).

Protection in chimeric TCR adenovirus-vaccinated mice was mediated by CD8+ T cells because treatment of vaccinated mice with an anti-CD8 mAb completely abrogated the protective effect (Fig. 5). This data are in agreement with our previous findings that protection can also be conferred by the adoptive transfer of T cells from TCR transgene by incorporating immunogenic T-helper epitopes, and to preserve the structure and conformation of the TCR. 

To date, tumor-derived TCR Id remains the only vaccine target for natural tumor Ag have been shown to be effective cancer vaccines in animal models (25, 26, 34), including our present report. Immunity to adenovirus may possibly prevent the effectiveness of repeated administration of adenovirus vaccines. However, several studies suggested that pre-existing immunity to adenovirus does not affect therapy. Adenovirus-mediated cancer therapy was not impaired in adenovirus-immune mice in one report (35). In addition, a Phase I clinical study reported that pre-existing humoral and cellular in patients did not preclude gene transfer (36). Furthermore, the barrier of pre-existing immunity can be overcome using a combination of adenovirus vaccine with various alternative forms of boosting, such as vaccination with a different adenovirus serotype (37), or adenovirus-infected dendritic cells (38, 39). Repeated immunization of adenovirus-infected dendritic cells has been shown to improve vaccine efficacy despite the presence of pre-existing antia adenovirus neutralizing Abs (40). The present form of adenovirus vaccines in our murine tumor model allows us to rapidly develop and compare the optimal vaccine design. Future studies will focus on further improving the efficacy of adenovirus vaccine by incorporating a boosting regimen in the vaccine protocol. In addition, the effectiveness of adenovirus vaccine in the treatment of mice with established tumor will be investigated to resemble the clinical situation.

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References

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