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Listeria-based HPV-16 E7 vaccines limit autochthonous tumor growth in a transgenic mouse model for HPV-16 transformed tumors

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Abstract

We have shown that *Listeria*-based cancer vaccines inhibit the growth of transplanted tumors in a transgenic mouse model of immune tolerance where HPV-16 E7 is expressed in the thyroid gland. In this study we determine whether these vaccines are able to inhibit autochthonous tumor growth in this animal model. Mice treated with *Listeria* vaccines expressing E7 had significantly smaller thyroid tumors than did mice treated with controls and possessed higher numbers of antigen-specific CD8+ T cells within the spleens, tumors, and peripheral blood. This study shows that *Listeria*-based vaccines are able to slow autochthonous tumor growth and break immunological tolerance.

Keywords

cancer vaccines; Listeria; HPV-16

Introduction

Human papillomavirus (HPV)-associated cancer is a significant cause of morbidity and mortality worldwide. Cervical cancer is the third most common cancer among women, and HPV-associated head and neck cancer affects over 100,000 people per year. [1,2] Although the newly approved prophylactic HPV vaccine should help decrease the incidence of cervical cancer in the future, millions of women who currently have cancer or have already been infected with HPV would benefit from a novel therapeutic HPV cancer vaccine. Unfortunately, cervical cancer patients often demonstrate decreased cytotoxic T-lymphocyte activity against HPV-associated antigens, which suggests they have acquired immunological tolerance.[3-5] Therefore, cancer vaccines designed to treat HPV-associated cancers must be able to overcome immunological tolerance in order to be effective.

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Disclosure: Yvonne Paterson wishes to disclose that she has a financial interest in Advaxis, Inc., a vaccine and therapeutic company that has licensed or has an option to license all patents from the University of Pennsylvania that concern the use of *Listeria* or listerial products as vaccines.

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Our laboratory has developed several HPV-specific recombinant *Listeria*-based vaccines which have been successful in pre-clinical models.[6-8] These *Listeria*-based vaccines act both as natural adjuvants and as HPV-specific vaccines which elicit a powerful, antigen-specific, cell-mediated immune response.[6,7] In order to test our vaccines' ability to elicit a productive cell mediated immune response in the face of immune tolerance, we developed a transgenic mouse which constitutively expresses the HPV-16 viral oncoproteins E6 and E7. E6 and E7 expression is controlled by the bovine thyroglobulin promoter and is localized to the thyroid gland. As a result, these mice are immunologically tolerant to E6 and E7.[8] In spite of this tolerance, our *Listeria* vaccines are able to generate an antigen-specific immune response sufficient to treat transplanted subcutaneous HPV-associated tumors in this model.[8]

Because of the transgene expression in the thyroid gland, our E6/E7 transgenic mice spontaneously develop thyroid tumors. Heretofore, we have only treated transplanted E7-expressing tumors with our *Listeria*-based vaccines, but not the thyroid tumors which spontaneously develop. These thyroid tumors are autochthonous and well-vascularized, and therefore provide another clinically relevant test for our vaccines. In this study, we test the ability of *Listeria*-based vaccines to elicit a tumor specific CTL response which can slow the growth of the autochthonous thyroid tumors generated in the transgenic mice. We hypothesize that *Listeria*-based vaccines will generate CD8⁺ tumor infiltrating lymphocytes and slow autochthonous tumor growth in the face of immunological tolerance. These studies represent the most rigorous model to date in which *Listeria*-based vaccines have been evaluated.

Materials and Methods

Mice

The E6/E7 transgenic mouse expresses HPV-16 E6 and E7 under the control of the thyroglobulin promoter and were developed as previously described.[8] Animals were bred, cared for and utilized in accordance with protocols approved by the Institutional Animal Care and Use Committee of The University of Pennsylvania (Philadelphia, PA) and the Philadelphia Veterans Administration Medical Center.

Listeria E7 Vaccines

Listeria based anti-E7 vaccines, Lm-LLO-E7 and Lm-ActA-E7, were used and maintained as previously described.[6,9] Lm-NP, a recombinant *Listeria* construct which expresses influenza nucleoprotein, was used as a negative control vaccine and maintained as previously described. [10]

Tumor Regression Study

Six- to 8-week old E6/E7 transgenic mice were divided into groups of ten or twelve mice which were then immunized intraperitoneally (i.p.) with 0.1 LD₅₀ Lm-LLO-E7, Lm-ActA-E7, Lm-NP, or left naive. The mice were immunized monthly for eight months. At the end of eight months, the thyroid tumors were excised and weighed. The average weights for each group of mice were statistically analyzed using one-way analysis of variance (ANOVA) when comparing more than two groups, and Student's *t* test when comparing two groups.

Splenic Analysis of E7-specific CD8+ T cells

Six- to 8-week-old E6/E7 transgenic mice were immunized i.p. with 0.1 LD₅₀ Lm-LLO-E7, Lm-ActA-E7, Lm-NP, or left untreated. Mice were boosted with the same vaccine dose monthly for eight months. One week after the final vaccination, mice were sacrificed, and spleens were pooled from 5 mice in each vaccination group and homogenized. Single cell suspensions were made for each group by filtration using a 100µm cell strainer (BD Biosciences

Pharmingen, San Diego, CA). Red blood cells (RBCs) were lysed using ACK Lysing Buffer (BioSource, Rockville, MD). Splenocytes were analyzed by four color flow cytometry on a FACScalibur for CD8 α (FITC), CD11b (PerCP Cy5.5), CD62L (APC) (BD Biosciences Pharmingen, San Diego, CA), 7AAD (Immunotech, Beckman-Coulter, Marseilles, FR), and tetramer (PE) of the H-2D^b restricted immunodominant E7 epitope in the C57BL/6 mouse (RAHYNIVTF). The E7/D^b tetramer was supplied by the NIAID Tetramer Core Facility at Emory University (Atlanta, GA) through the NIH AIDS Research and Reference Reagent Program. Cells were analyzed by comparing tetramer⁺, CD8⁺, CD11b⁻, 7AAD⁻, and CD62L^{low} cells within the spleens of the different groups. Data were analyzed with CellQuest software (BD biosciences).

ELISPOT analysis of PBMCs and thyroid infiltrating lymphocytes

To prepare PBMCs, whole blood was collected from 5 mice per vaccination group with sodium heparin (18 units/ml) one week after the final immunization. Erythrocytes were lysed with ACK lysing buffer (BioSource) and washed twice in RP-10. The cell suspensions were then separated over Ficoll-hypaque (Amersham Biosciences) in DMEM.

To prepare tumor infiltrating lymphocytes, thyroids from 10 mice per vaccination group were harvested one week after the final immunization, pooled and homogenized in RP-10 using nylon mesh bags followed by filtration through a cell strainer (BD Biosciences Pharmingen). Erythrocytes were lysed using ACK lysing buffer and washed twice in RP-10. The cell suspensions were separated in a Ficoll-hypaque gradient in DMEM.

ELISPOT was performed using IFN-γ reagents from MabTech (Sweden) and nitrocellulose plates from Cellular Technology (Cleveland, OH) or Millipore (Billerica, MA). Briefly, the 96-well filtration plates were coated with 7.5μg/ml rat anti-mouse IFN-γ antibody (clone AN18, MABTECH, Mariemont, OH) in 100μl of PBS. After overnight incubation at 4°C, the wells were washed and blocked with culture medium containing 10% fetal bovine serum. Serial dilution of these suspensions starting with PBMCs 2×10⁵ cells/well and thyroid cells suspensions 1×10^6 cells/well were added to each well along with peptide representing the E7specific CTL epitope (5µg/ml) plus IL-2 (5U/ml). Concanavalin A was added to positive control wells instead of peptide, and distilled water was added to negative control wells instead of the peptide. Cells were incubated at 37°C for 24 hrs. The plate was then washed, followed by incubation with 1µg/ml biotinylated IFN-γ antibody (clone R4-6A2, MABTECH) in 100ul of PBS at 4°C overnight. After washing, 1:1000 strepavidin-horseradish peroxidase in 100µl PBS was added and incubated at room temperature for 1 hr. Spots were developed by adding 100µl of TMP substrate and incubated at room temperature for 5-10min. The color development was stopped by washing extensively in tap water. The spots were counted on an ELISPOT reader and reported as spots per 10⁶ cells. After drying, the plates were scanned and counted using an ELISPOT reader (Cellular Technology Ltd, Cleveland, OH). A positive response was defined as 50 spot forming units (SFU) per million peripheral blood mononuclear cells (PBMCs). The background observed in this study was below 20 SFU per million PBMCs.

Measurement of E7 antibody titers by ELISA

10 mice per group of wild type mice (C57BL/6) or E6/E7 transgenic mice were immunized monthly with 0.1 LD50 of Lm-LLO-E7, Lm-ActA-E7, or Lm-LLO-NP. Serum samples were harvested on day 7 after the 8th immunization and diluted serially in PBS.

96 well plates were coated with 50 µl per well with a solution of 15µg/ml of E7 protein, and blocked with 5% FCS in PBS for 1hr at room temperature. The E7 protein was expressed using the pQE (Qiagen) vector expression system and purified in the following manner. Bacterial pellets were resuspended and lysed by sonication in Tris-buffered saline (TBS) containing 1%

Triton X-100 and 10 mM imidazole. Debris was removed by centrifugation. Ni²⁺-NTA– agarose beads (Qiagen) were added and incubated with the lysate for 30 min. Beads were washed extensively and protein was eluted with TBS containing 150 mM imidazole.

Mouse sera samples were diluted to 1 to 100 and then serially diluted by 1 to 4. These were added to the plates and incubated for 1hr at room temperature. Then, anti-mouse IgG1 HRP was added (Amersham Bioscience). Subsequently, the chromagen ABTS was added and plates were read at A405. Statistical analysis using one-way ANOVA and the paired Student's T-test was performed.

Results

Lm LLO-E7 and Lm-ActA-E7 limit the size of autochthonous thyroid tumors

The vaccines Lm-LLO-E7 and Lm-ActA-E7 were tested for their ability to reduce the size of autochthonous thyroid tumors in our transgenic mouse model. Beginning at 6-8 weeks of age, the vaccines were injected i.p. into 10 or 12 transgenic mice at monthly intervals. Injections continued for 8 months. As control groups, ten additional mice were vaccinated with a *Listeria* vaccine which expresses an irrelevant influenza nucleoprotein (Lm-LLO-NP), and an additional group of 10 mice was left unvaccinated. One week after the final vaccination, the thyroid glands were harvested and weighed.

The experiment was performed twice (Figure 1, Table I). In the first experiment, the average thyroid mass was over 500 mg in both control groups, while the average mass was 239 mg in the Lm-LLO-E7 group and 276 in the Lm-ActA-E7 group. These were statistically significant differences (P<0.001, one-way ANOVA and Student's *t* test). In the second experiment, the average thyroid masses of the control groups (584 mg for naive and 367 mg for Lm-LLO-NP) was significantly higher than the experimental Lm-LLO-E7 and Lm-ActA-E7 groups (220 and 276, respectively, P<0.001 one-way ANOVA and Student's *t* test). Interestingly, in this experiment the group of mice treated with the non-antigen specific control vaccine Lm-NP had reduced tumor burden compared to untreated animals (367 and 584, respectively, P< 0.0001, Student's *t* test).

Lm-LLO-E7 and Lm-ActA-E7 stimulate E7-specific CD8 lymphocytes in the spleens of transgenic mice

In order to detect E7-specific, activated CD8+ lymphocytes in vaccinated transgenic mice, splenocytes were harvested one week after the last of 8 monthly vaccinations. The splenocytes were harvested from 5 mice in each treatment group. The splenocytes were stained with antibodies to CD8 and CD62L, and then were stained with the H-2D^b tetramer loaded with the E7 peptide RAHYNIVTF. The percentage of activated CD62L^{lo}, CD8+, tetramer positive cells from each group was determined by flow cytometry. Groups treated with Lm-LLO-E7 and Lm-ActA-E7 had higher percentages of E7-specific lymphocytes by 3-4 fold over controls (Figure 2).

Lm-LLO-E7 and Lm-ActA-E7 stimulate E7-specific CD8 lymphocytes in the thyroid glands of transgenic mice

We hypothesized that the administration of our vaccines would also lead to E7-specific lymphocytes within the thyroid glands of transgenic mice. In order to test this, thyroid glands were harvested from mice one week after 8 monthly vaccinations. After the sizes and weights were determined, the thyroid glands were digested to create single-cell suspensions, then stained with antibodies to CD8, CD62L and H-2D^b tetramer. We found few E7 specific lymphocytes within the thyroid glands of untreated naïve mice (0.07% of activated CD8+ lymphocytes within the thyroid) and mice treated with the control vaccine Lm-LLO-NP

(0.23%). In contrast, larger percentages were seen in mice vaccinated with the experimental vaccines Lm-LLO-E7 (5.12%) and Lm-ActA-E7 (1.41%) (Figure 3).

Lm-LLO-E7 and Lm-ActA-E7 generate an increased percentage of E7-specific lymphocytes in the peripheral blood of transgenic mice

In order to determine whether our vaccines generated circulating antigen specific T cells, peripheral blood was drawn from the tails of transgenic mice after 8 months of vaccine treatments. PBMCs were stained with antibodies to CD8, CD62L and H-2D^b tetramer as described previously. We found very few circulating E7-specific T cells within the blood of untreated transgenic mice. However, in mice vaccinated with either Lm-LLO-E7 or Lm-ActA-E7, there was a dramatic 10-20 fold increase in the percentage of activated peripheral blood CD8 cells which were specific for E7 in transgenic mice (Figure 4).

E7-specific lymphocytes in the peripheral blood and thyroid tumors secrete increased amounts of IFN- γ

We sought to confirm the ability of the E7-specific lymphocytes generated by our vaccines to secrete IFN- γ in response to the E7 H-2D^b – restricted epitope. We first tested lymphocytes in the peripheral circulation. After 8 months of vaccinations, peripheral blood was drawn and PBMCs were isolated. Following this, thyroids were harvested and mononuclear cells separated by Ficoll-hypaque. Cells were pooled from ten mice for subsequent ELISPOT analysis. ELISPOT analysis was performed as described in the Materials and Methods, using the H-2D^b epitope RAHNITVYF as the stimulatory peptide. As detailed in Figure 5a, there were a significantly higher number of spot forming colonies (SFC) in mice vaccinated with Lm-LLO-E7 and Lm-ActA-E7 than in naïve mice or those vaccinated with Lm-LLO-NP. (p<0.0001, one-way ANOVA, Figure 5a). We then performed ELISPOT analysis on the lymphocytes isolated from the thyroid gland of transgenic mice. Again we found a statistically significant difference in the number of SFC in the groups vaccinated with Lm-LLO-E7 and Lm-ActA-E7 compared with negative controls (Figure 5b).

Humoral responses to vaccination with Lm-LLO-E7 and Lm-ActA-E7

In order to determine whether vaccination with Listeria vaccine constructs resulted in increased E7-specific antibody production, ELISAs were performed on sera from both wild-type and transgenic mice. The serum samples were collected one week after the last of 8 monthly vaccinations with either Lm-LLO-E7, Lm-ActA-E7, or Lm-LLO-NP. Serum from a fourth, untreated group was also tested.

In wild-type mice, there was an increase in E7-specific antibody production in mice vaccinated with Lm-LLO-E7 and Lm-ActA-E7 over negative controls at the first (1 to 100) and second (1 to 400) serum dilutions (p<0.0001 and p<0.0004, respectively, one-way ANOVA). The difference in absorbance readings was not significant at the remaining dilutions (Figure 6a). In transgenic mice, antibody production was highest in mice vaccinated with Lm-LLO-E7, followed by Lm-ActA-E7, then the negative controls (Figure 6b). Sera from mice vaccinated with Lm-LLO-E7 had significantly higher absorbance readings at each of the first four serial dilutions (P<0.001, one-way ANOVA) indicating a titer of 1 to 6,400. In addition, absorbance readings were generally higher in the transgenic mice in comparison to wild-type mice (peak absorbance in trangenics 1.43 compared to 0.635 ODU in wild-type).

Discussion

We have demonstrated the ability of *Listeria*-based vaccines to slow tumor growth and break immunological tolerance in an autochthonous tumor model. In our previous study with this model, we showed *Listeria*-based vaccines were able to cause the complete regression of

transplantable subcutaneous HPV-associated tumors in some mice.[8] In the present study, we show efficacy of the vaccines against autochthonous tumors, which are more difficult to treat. Autochthonous thyroid tumors have a much more robust blood supply than the subcutaneous tumors, and the thyroid undergoes neoplastic changes weeks before the first vaccinations are administered. This autochthonous model is relevant to the clinical situation faced in HPV-associated cervical or head and neck cancer patients, where the tumors are very well-vascularized and have been present for months to years before the diagnosis is made and treatment begins.

Among the most encouraging results we found was not only the decrease in tumor size, but also the increased secretion of IFN- γ from both tumor infiltrating and peripheral lymphocytes. In cervical cancer patients, it has been shown that there is deviation to a Th2 immune response in the periphery.[11] Thus, one of the objectives of therapeutic immunotherapy will be to generate a Th1 response in these patients and to reverse the Th1/Th2 balance. In the transgenic animals, there was increased secretion of the Th1 cytokine IFN- γ in the peripheral blood as a result of vaccination with Lm-LLO-E7 and Lm-ActA-E7. The ability of these vaccines to generate a Th1 response makes them attractive candidates for clinical trials for cervical cancer.

When comparing the results from our two therapeutic vaccines, Lm-LLO-E7 and Lm-ActA-E7, we showed that both vaccines reduced tumor sizes to a similar degree. In addition, the percentages of E7-specific T cells were similar in both the peripheral blood and in the spleen. However, our tetramer and ELISPOT data indicates Lm-LLO-E7 induced many more E7-specific T cells within the thyroid itself. Interestingly, this marked increase in antigen-specific TILs in Lm-LLO-E7 vaccinated mice did not lead to a similar reduction in tumor size when compared to mice vaccinated with Lm-ActA-E7. This result seems to indicate that there is a threshold of antigen specific TILs, over which there is no added therapeutic benefit. The reasons for this are unclear. The presence of regulatory T cells is most likely not a factor, as we have shown in a previous study that the depletion of regulatory T cells did not affect vaccine efficacy. [8] When interpreting the tetramer data, it is important to note that the immunological assays were performed after eight months of vaccinations, and that numbers of antigen-specific cells may have varied over the course of the experiment. The number of TILs in the ActA vaccinated mice may have been the same or higher at other time points. Future studies will be designed to examine the variation in antigen-specific T cells over time.

While we observed a significant reduction in tumor size, there was not complete shrinkage of the thyroids in these mice. This is in contrast to our previous study, where complete regression of subcutaneous transplanted tumors was seen in roughly 25% of mice.[8] Future modifications to improve our vaccine strategy may include increasing the frequency of vaccine administration. Although we detected significant numbers of circulating and tumor infiltrating lymphocytes one week after vaccination, one can imagine these numbers may dwindle significantly during the remaining three weeks before another vaccination is administered. The number of mice available hindered our ability to harvest samples on a weekly basis in this study. However, future experimental design will include both monthly and weekly vaccinations, and performing immunological analysis more frequently to determine the optimal dosing strategy.

It is of interest that in one experiment treatment of transgenic mice with the control vaccine Lm-NP had a therapeutic effect suggesting that, although *Listeria* is gram positive and lacks LPS, it can act as a "Coley's toxin". These findings are consistent with observations by us and others that *Listeria* can slow the growth of experimental metastases of B16F10 melanoma in the lung[12] and of CT26 colorectal tumors in the liver[13] without expressing the appropriate tumor antigen. This is most likely because *Listeria* can induce an early innate immune response followed by an adaptive Th1 response, which results in the secretion of TNF-α, IFN-γ, and

IL-2 from activated lymphocytes.[14-17] This cellular immune response to *Listeria* may result in the non-specific tumor responses demonstrated in our study.

The transgenic mice we developed express both E6 and E7 proteins. Although it is conceivable that a vaccine strategy which targets both antigens would increase therapeutic efficacy, we have focused on an E7-based strategy. E7 is the antigen targeted in the vast majority of clinical trials for HPV-associated cancer, and our E7-based *Listeria* vaccines have been far more effective than our E6-based vaccines in previous experiments in mice. Furthermore, the addition of an E6 vaccine did not improve the tumor responses in an implantable mouse tumor model (unpublished observations). As a proof of principle, we have focused solely on E7 as a target antigen in this study.

Although our *Listeria*-based vaccines are designed to maximize the cellular immune response, humoral immune responses were detected in vaccinated mice. Interestingly, antibody production was higher in transgenic mice than wild-type mice as determined by spectrophotometer analysis after ELISA. This may be the result of increased amounts of E7-protein in the periphery due to the production of E7 in the thyroid gland and the lysis of thyroid cells. However, because E7 is not a cell surface protein, it is unlikely that the humoral immune response contributes significantly to the reduction in tumor size in the transgenic mice.

Lastly, the safety of our attenuated bacterial vaccines is further documented with this study. The animal subjects received 8 doses of recombinant *Listeria*, which is the most administered in any pre-clinical or clinical trial to date. Of the 80 animals tested, none had adverse side effects such as cachexia or failure to thrive, nor did they show any signs of listeriosis. When administered appropriately in the correct dosage, Listeria based vaccines are both efficacious and safe in our pre-clinical models.

In conclusion, *Listeria*-based vaccines can slow tumor growth and break immunological tolerance in an autochthonous tumor model. This study, among others, contributes to the growing body of literature supporting the use of recombinant bacterial vaccines in human clinical trials for cancer.

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Abbreviations

PBMC

peripheral blood mononuclear cells

HPV

Human Papillomavirus

TILs

tumor infiltrating lymphocytes

Lm

Listeria monocytogenes

LLO

Listeriolysin O

CTL

cytotoxic T lymphocytes

RBC

red blood cells

ActA

actin assembly

ODU

optical density unit

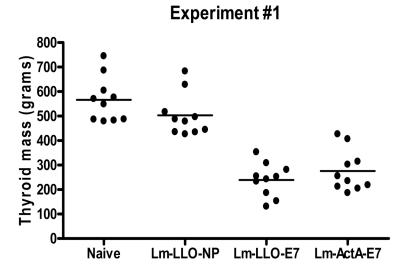
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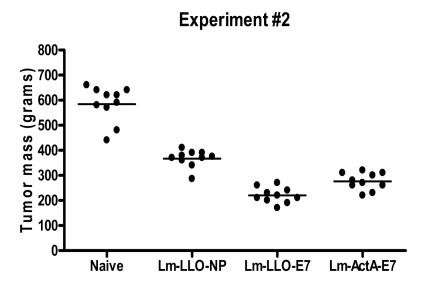


Figure 1. Reduction in thyroid gland mass after vaccinations with *Listeria***-based vaccines** After 8 months of vaccinations with Listeria-based vaccines, thyroid glands from transgenic mice were harvested and measured. Individual sizes from each of the four groups are shown. The experiment was performed twice. In both experiments, the difference in average sizes between each of the experimental groups (Lm-ActA-E7 and Lm-LLO-E7) and the negative control groups (naïve and Lm-LLO-NP) is statistically significant (P<0.001, one-way ANOVA).

Tetramer analysis: Splenocytes

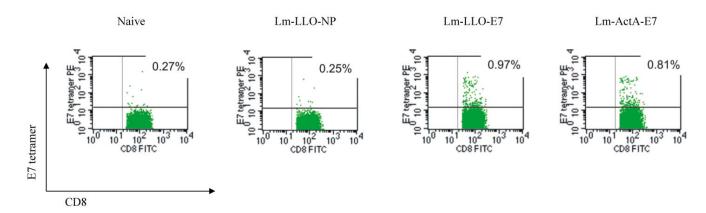


Figure 2. Tetramer analysis of E7-specific lymphocytes in the spleens of vaccinated transgenic mice Splenocytes were pooled from 5 mice in each vaccine group one week after 8 months of monthly vaccinations. They were stained with antibodies to CD8, CD62L, and E7/D^b tetramer and the data analyzed by flow cytometry. The experiment was performed twice. Shown are representative plots from one experiment. These data show that Lm-LLO-E7 and Lm-ActA-E7 generate increased numbers of E7-specific lymphocytes in the spleens of transgenic animals when compared to controls.

Tetramer analysis: Thyroid TILs

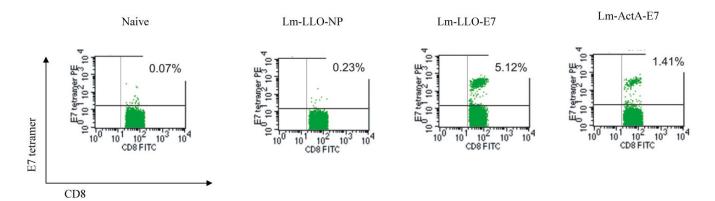


Figure 3. Tetramer analysis of E7-specific TILs from the thyroid glands of transgenic mice Single cell suspensions from thyroids were pooled from 5 mice in each vaccine group one week after 8 months of monthly vaccinations. They were stained with antibodies to CD8, CD62L, and E7/D^b tetramer and the data analyzed by flow cytometry. The experiment was performed twice. Shown are representative plots from one experiment. These data show that Lm-LLO-E7 and Lm-ActA-E7 administration stimulates E7-specific Tlymphocytes in the thyroid gland.

Tetramer analysis: PBMCs

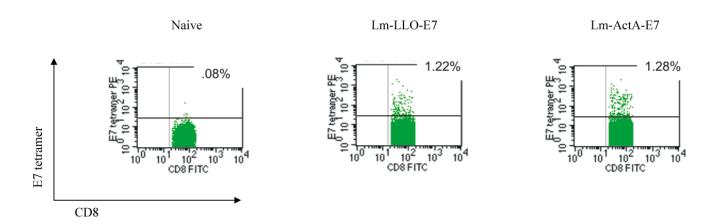


Figure 4. Tetramer analysis of E7-specific lymphocytes PBMC from vaccinated transgenic mice PMBCs from 5 mice in each vaccine group one week after 8 months of monthly vaccinations. They were stained with antibodies to CD8, CD62L, and E7/D^b tetramer and the data analyzed by flow cytometry. The experiment was performed twice. Shown are representative plots from one experiment. These data show that Lm-LLO-E7 and Lm-ActA-E7 administration stimulates E7 specific T lymphocytes in the peripheral blood.

Figure 5a

ELISPOT assay: PBMCs

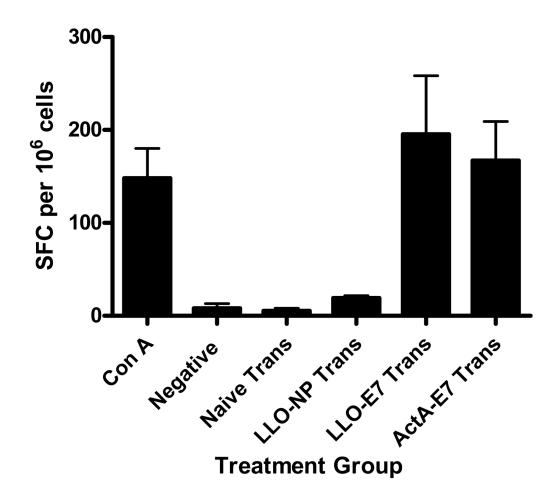


Figure 5b

ELISPOT assay: TILs

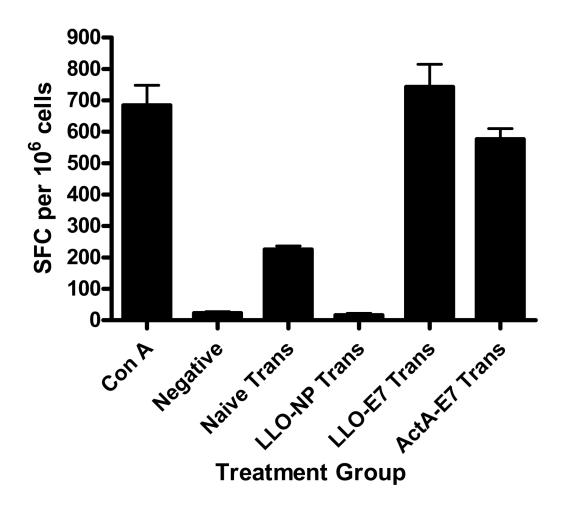


Figure 5. ELISPOT assay of PBMCs and TILs from transgenic mice PBMC (a) and thyroid infiltrating lymphocytes (b) from vaccinated transgenic mice were harvested one week after the eighth and final vaccination with the *Listeria* constructs, and standard IFN- γ ELISPOT assays were performed. Mice vaccinated with Lm-LLO-E7 and Lm-ActA-E7 demonstrated significantly more spot forming colonies than controls (p<0.0001, one-way ANOVA). SFCs = spot-forming colonies.

Figure 6a

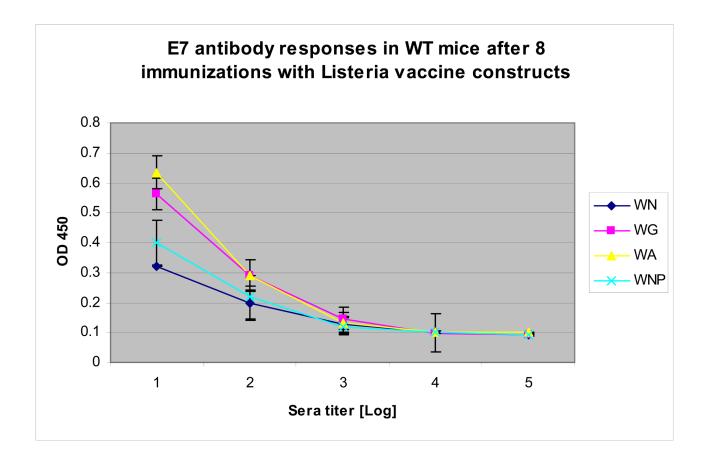


Figure 6b

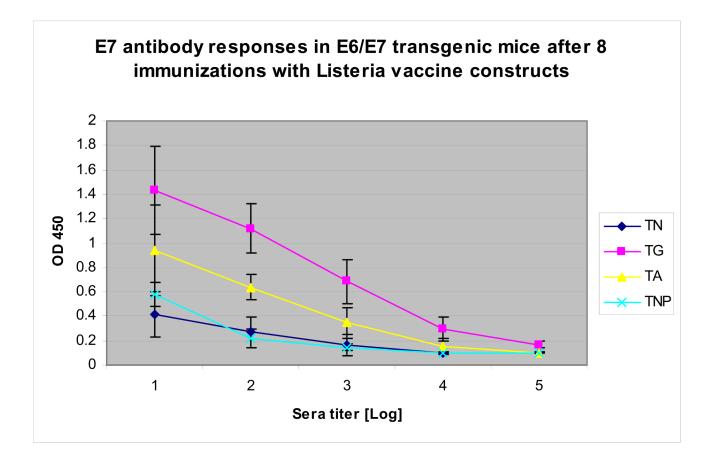


Figure 6. E7-specific antibody production in wild-type and transgenic mice after vaccination Serum samples were harvested one week after the eighth immunization and diluted serially in PBS and standard ELISA assays were performed as described. The y axis represents $100 \times \text{Log}_4$ dilutions. In both wild type (a) and transgenic (b) mice, statistically significant increases in E7-specific antibody production were seen in Lm-LLO-E7 and Lm-ActA-E7 vaccinated mice (p<0.0001, one way ANOVA). W=wild type mice, T=transgenic: N=naïve; G=Lm-LLO-E7; A= Lm=ActA-E7; NP=Lm-LLO-NP

Table IReduction in thyroid gland mass after 8 vaccinations with *Listeria*-based vaccines

	Naive	Lm-LLO-NP	Lm-LLO-E7	Lm-ActA-E7
Thyroid mass, Experiment 1 (mg)	566.3 ± 91.6	502.8 ± 86.9	239 ± 68	275.8 ± 84.5
Thyroid mass, Experiment 2	584 ± 71.8	367 ± 34.1	220 ± 30.9	276 ± 34.4