Recombinant Listeria promotes tumor rejection by CD8+ T cell-dependent remodeling of the tumor microenvironment.

Title
Recombinant Listeria promotes tumor rejection by CD8+ T cell-dependent remodeling of the tumor microenvironment.

Permalink
https://escholarship.org/uc/item/4zw9294w

Journal
Proceedings of the National Academy of Sciences of the United States of America, 115(32)

ISSN
0027-8424

Authors
Deng, Weiwen
Lira, Victor
Hudson, Thomas E
et al.

Publication Date
2018-08-01

DOI
10.1073/pnas.1801910115

Peer reviewed
Recombinant *Listeria* promotes tumor rejection by CD8\(^+\) T cell-dependent remodeling of the tumor microenvironment

Weiven Deng\(^{a,1}\), Victor Lira\(^a\), Thomas E. Hudson\(^a\), Edward E. Lemmens\(^a\), William G. Hanson\(^a\), Ruben Flores\(^a\), Gonzalo Barajas\(^a\), George E. Katibah\(^a\), Anthony L. Desbiens\(^a\), Peter Lauer\(^a\), Meredith L. Leong\(^a\), Daniel A. Portnoy\(^bc\), and Thomas W. Dubensky Jr.\(^{a,1,2}\)

*Aduro Biotech, Inc., Berkeley, CA 94710; \(^a\)Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720; and \(^b\)The School of Public Health, University of California, Berkeley, CA 94720

Edited by Harvey Cantor, Dana-Farber Cancer Institute, Boston, MA, and approved June 29, 2018 (received for review February 6, 2018)

Agents that remodel the tumor microenvironment (TME), prime functional tumor-specific T cells, and block inhibitory signaling pathways are essential components of effective immunotherapy. We are evaluating live-attenuated, double-deleted *Listeria monocytogenes* mono- cytogenes expressing tumor antigens (LADD-Ag) in the clinic. Here we show in numerous mouse models that while treatment with nonrecombinant LADD induced some changes in the TME, no antitumor efficacy was observed, even when combined with immune checkpoint blockade. In contrast, LADD-Ag promoted tumor rejection by priming tumor-specific KLRG1\(^+\)PD1\(^+\)CD62L\(^−\) CD8\(^+\) T cells. These IFN\(γ\)-producing effector CD8\(^+\) T cells infiltrated the tumor and converted the tumor from an immunosuppressive to an inflamed microenviron- 

*Listeria monocytogenes* \(\rightarrow\) CD8\(^+\) T \(\rightarrow\) tumor microenvironment \(\rightarrow\) cancer vaccine

Under immunosuppressive tumor microenvironment (TME) conditions, tumor-specific T cells rapidly become exhausted (1). These dysfunctional T cells express high levels of inhibitory receptors (e.g., PD1, LA3G) and are impaired in their ability to produce cytokines such as TNF\(\alpha\) and IFN\(γ\) (2). Suppressive leukocyte populations in the TME that inhibit T cell function include regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and tumor-associated macrophages (TAMs) (1). TAMs have distinct functional types ranging from classically activated macrophages (M1) associated with acute inflammation and functional T cell immunity, to immunosuppressive macrophages (M2) associated with promoting tumor proliferation (3). Of those genes that define M2 macrophages, arginase 1 (Arg1) and mannose receptor (CD206) led to the concept of alternative activa- 

Conflict of interest statement: W.D., V.L., T.E.H., E.E.L., R.F., G.B., and A.L.D. performed research; W.G.H., P.L., and D.A.P. designed and constructed LADD strains; W.D., V.L., T.E.H., E.E.L., and G.E.K. analyzed data; and W.D. and T.W.D. wrote the paper.

Significance

The development of therapeutic cancer vaccines using recombinant microorganisms has been pursued for many decades. However, the underlying mechanisms of therapeutic cancer vaccines remain unclear. Here we compare recombinant *Listeria*-based cancer vaccines to synthetic long peptide and adenovirus delivery systems for tumor antigens, and describe immunologic correlates of antitumor efficacy of *Listeria*-based cancer vaccines. Our results show that the profound antitumor efficacy requires tumor microenvironment (TME) remodeling that depends on tumor-specific CD8\(^+\) T cells induced by live-attenuated double-deleted *Listeria monocytogenes* expressing cognate tumor antigens. Together, this work highlights the importance of cognate tumor antigen expression by cancer vaccines and pinpoints the relationship between induced tumor antigen-specific immunity and the TME.

Author contributions: W.D., M.L.L., and T.W.D. designed research; W.D., V.L., T.E.H., E.E.L., R.F., G.B., and A.L.D. performed research; W.G.H., P.L., and D.A.P. designed and constructed LADD strains; W.D., V.L., T.E.H., E.E.L., and G.E.K. analyzed data; and W.D. and T.W.D. wrote the paper.

Data deposition: The raw sequence data reported in this manuscript have been deposited in the National Center for Biotechnology Information Sequence Read Archive (https://www.ncbi.nlm.nih.gov) with accession nos. SRK7271231, SRK7271232, SRK7271229, SRK7271230, and SRK7271233.

1To whom correspondence may be addressed. Email: wdeng@aduro.com or tdubensky@aduro.com

2Present address: Tempest Therapeutics, San Francisco, CA 94104.

This article is a PNAS Direct Submission. This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).
LADD-Ag administration inhibits growth of multiple tumor models in a CD8

8) treated with HBSS or LADD-AH1 on day 7. On days 6 and 8, 200

C

0.0001. Results are representative of CT26 in BALB/c mice (SI Appendix, Fig. S1A) (16), which allowed us to test LADD-AH1 efficacy in BALB/c mice bearing these tumors. A single i.v. injection of LADD-AH1 induced complete CT26 tumor rejection in six of eight mice (Fig. 1A); however, no reduction in tumor growth was observed with LADD treatment. Notably, i.v. injection of adenovirus-expressing AH1 (Ad-AH1) did not control CT26 tumor growth. Consistently, LADD-AH1 induced robust peripheral AH1 responses, whereas LADD and Ad-AH1 induced poor AH1 responses (Fig. 1B). Similarly, LADD-AH1 treatment inhibited 4T1 tumor growth (Fig. 1C). Tumor growth was not inhibited in LADD-treated mice, even when combined with PD-1 blockade (Fig. 1C). In contrast, LADD-AH1 combined with α-PD-1 resulted in complete 4T1 tumor eradication (Fig. 1C).

Tumor-specific CD8+ T cells were critical for LADD-AH1 efficacy, as control of CT26 tumor growth was largely lost by CD8+ T cell depletion (Fig. 1D), which also prevented peripheral AH1 responses (SI Appendix, Fig. S1B). In contrast, depletion of CD4+ T cells did not impact LADD-AH1 efficacy (SI Appendix, Fig. S1C). An adoptive transfer experiment further confirmed this.

**Results**

**LADD-Ag Administration Inhibits Tumor Growth in a CD8+ T Cell-Dependent Manner.** To test efficacy and dissect the mechanism of action of antitumor responses, we constructed a LADD strain that expresses AH1 (LADD-AH1). AH1 is a dominant H-2Ld-restricted CD8+ T cell epitope derived from endogenous retroviral antigen gp70 (15). This antigen is expressed in both CT26 and 4T1 tumor cells (SI Appendix, Fig. S1A) (16), which allowed us to test LADD-AH1 efficacy in BALB/c mice bearing these tumors. A single i.v. injection of LADD-AH1 induced complete CT26 tumor rejection in six of eight mice (Fig. 1A); however, no reduction in tumor growth was observed with LADD treatment. Notably, i.v. injection of adenovirus-expressing AH1 (Ad-AH1) did not control CT26 tumor growth. Consistently, LADD-AH1 induced robust peripheral AH1 responses, whereas LADD and Ad-AH1 induced poor AH1 responses (Fig. 1B). Similarly, LADD-AH1 treatment inhibited 4T1 tumor growth (Fig. 1C). Tumor growth was not inhibited in LADD-treated mice, even when combined with PD-1 blockade (Fig. 1C). In contrast, LADD-AH1 combined with α-PD-1 resulted in complete 4T1 tumor eradication (Fig. 1C).

Tumor-specific CD8+ T cells were critical for LADD-AH1 efficacy, as control of CT26 tumor growth was largely lost by CD8+ T cell depletion (Fig. 1D), which also prevented peripheral AH1 responses (SI Appendix, Fig. S1B). In contrast, depletion of CD4+ T cells did not impact LADD-AH1 efficacy (SI Appendix, Fig. S1C). An adoptive transfer experiment further confirmed this.

**LADD-Ag Immunotherapy Induces Splenic Tumor-Specific CD8+ Effector T Cells.** To determine whether Ag expression by LADD affected the cytokine profile, we measured serum cytokines induced by LADD and LADD-AH1 in CT26 tumor-bearing mice. At 6-h postinjection, LADD and LADD-AH1 induced comparable proinflammatory cytokines including MCP-1, IL-12p70 and IFNγ, demonstrating that Ag expression does not alter cytokine induction (SI Appendix, Fig. S2A). To assess possible functional differences, we performed RNA-Seq analysis of peripheral CD8+ T cells purified from CT26 tumor-bearing mice that were treated with LADD or LADD-AH1. Principal component analysis indicated that peripheral CD8+ T cells from HBSS, LADD, or LADD-AH1–treated mice exhibited distinct transcriptional features (SI Appendix, Fig. S2B). Remarkably, peripheral CD8+ T cells from LADD-AH1–treated mice exhibited increased expression of genes related to
T cell activation and cytotoxicity signatures, including a substantial increase in expression of Tbx21, Ifng, Gzmb, Cxcl1, and Kift1, relative to HBSS and LADD treatment (Fig. 2A and SI Appendix, Table S1). The magnitude of the peripheral AH1-specific CD8\(^+\) T cell population also increased dramatically after LADD-AH1 treatment relative to LADD (Fig. 2B). This T cell population largely displayed a short-lived effector phenotype, characterized by CD44\(^+\)CD62L\(^-\) KLRG1\(^+\), consistent with RNA-Seq analysis (Fig. 2 C and D). While CD44\(^+\)CD62L\(^-\) CD8\(^+\) T cells trended higher with LADD treatment relative to HBSS control mice, this difference was not significant (Fig. 2C). Notably, most of AH1-specific CD8\(^+\) T cells induced by LADD-AH1 were KLRG1\(^+\)CD62L\(^-\)CX3CR1\(^-\)CD44\(^+\) (SI Appendix, Fig. S2 C-F).

Recent results from two clinical trials highlight the potential of targeting tumor-specific neoantigens with a therapeutic vaccine (18, 19). To test whether LADD can elicit neoantigen-specific CD8\(^+\) T cells, we constructed an LADD strain expressing MC38 neoepitopes Adpgk and Reps1 (LADD-Neo) (20). Robust Adpgk\(^+\)CD8\(^+\) T cells were detected by tetramer staining in MC38 tumor-bearing mice treated with LADD-Neo (SI Appendix, Fig. S3 A and B). Significantly, most of the LADD-Neo-induced Adpgk\(^+\)CD8\(^+\) T cells were also KLRG1\(^+\) (SI Appendix, Fig. S3B). LADD-Neo induced significant responses against the Adpgk neoepitope but did not cross-react against its native sequence, and LADD-Neo induced higher Adpgk responses in tumor-bearing mice than in naive mice (SI Appendix, Fig. S3C).

To identify locations of tumor-specific CD8\(^+\) T cell priming, immune responses in tumor draining lymph nodes (TDLN) and spleen were measured 4 d after LADD-AH1 treatment. TDLN from LADD-AH1–treated mice exhibited enhanced AH1 responses relative to those from HBSS or LADD treatment (Fig. 2E). Additionally, AH1 responses were observed in tumor-bearing but not naive mice, indicating that implanted CT26 cells primed AH1-specific responses (Fig. 2E). The splenic AH1 CD8\(^+\) T cell response induced by LADD-AH1 was significantly higher than the response measured in the TDLN, which we confirmed by AH1 tetramer analysis (Fig. 2 F and G). These results indicated that AH1-specific CD8\(^+\) T cells localized preferentially to the spleen and did not reside in the TDLN, likely due to a lack of CD62L expression (SI Appendix, Fig. S2D). Consistent with their predominant splenic localization, LADD-AH1-induced splenic CD8\(^+\) T cells displayed increased Ki67, a proliferation marker (Fig. 2G). Furthermore, splenic AH1\(^+\)CD8\(^+\) T cells induced by LADD-AH1 in CT26 tumor-bearing mice produced IFN\(\gamma\) in response to CT26 as well as 4T1 cells stimulation ex vivo (Fig. 2H and SI Appendix, Fig. S4 A and B). This was not surprising as both CT26 and 4T1 express Gp70 (SI Appendix, Fig. S1A). However, LADD-AH1 treatment failed to induce responses against CT26 neoepitopes whose vaccination were reported to reject CT26 tumor (21) (SI Appendix, Fig. S4C).

However, these data do not exclude the possibility of epitope spreading upon LADD-Ag to unknown CT26 antigens. Notably, splenic AH1\(^+\)KLRG1\(^+\) CD8\(^+\) T cells were strongly induced by LADD-AH1 treatment, whereas these cells existed at low levels in LADD and HBSS groups (Fig. 2I). To explore whether AH1\(^+\)KLRG1\(^+\) CD8\(^+\) T cells were differentiated and expanded from AH1\(^+\)KLRG1\(^+\) CD8\(^+\) T cells primed by tumor implantation, splenic CD45.1\(^+\)CD8\(^+\) T cells from tumor-bearing mice were adoptively transferred into naive CD45.2\(^+\) recipients. Indeed, donor AH1\(^+\)KLRG1\(^+\) CD8\(^+\) T cells expanded and differentiated into AH1\(^+\)KLRG1\(^+\) CD8\(^+\) T cells upon LADD-AH1 but not LADD immunization (Fig. 2J and SI Appendix, Fig. S4D). While an increase in KLRG1 expression and proliferation of donor CD8\(^+\) T cells was observed in recipients upon LADD immunization, this strain did not expand AH1\(^+\) CD8\(^+\) T cells, demonstrating a requirement for LADD-mediated expression of the cognate tumor Ag (SI Appendix, Fig. S4D). Recipient CD45.2\(^+\)AH1\(^+\) CD8\(^+\) T cells were detectable but at a low frequency following LADD-AH1 injection (Fig. 2J), consistent with a weak tumor-specific response in naive animals (Fig. 1G and SI Appendix, Fig. S3C).

**LADD-Induced Functional Tumor-Specific CD8\(^+\) T Cells Infiltrate into Tumors.** TME modification to promote tumor cell destruction in malignancies is an essential step for effective immunotherapy. We measured the magnitude and interrogated the phenotype of CD8\(^+\) T cells infiltrating CT26 tumors to characterize how LADD impacts the TME, and to determine whether LADD-induced tumor-specific CD8\(^+\) T cells affected the TME profile. Immunohistochemistry (IHC) and flow cytometry analysis 4 d after LADD-AH1 treatment showed a marked increase in the frequency of tumor

---

**Fig. 2.** LADD-AH1 immunotherapy induces splenic AH1-specific CD8\(^+\) effector T cells. (A–J) CT26 tumor-bearing BALB/c mice (n = 8) were i.v. injected with HBSS, LADD, or LADD-AH1 on day 7. (A–D) On day 14, (A) RNA-Seq analysis of peripheral CD8\(^+\) T cells purified from a pool of eight mice per group. Heat map of selected genes from the core signature. Log\(_2\) of gene expression values (TPM, transcripts per million) are colored from blue to red. Frequencies of (B) AH1 tetramer\(^*\), (C) CD44\(^+\)CD62L\(^-\), and (D) KLRG1\(^+\) of peripheral CD8\(^+\) T cells were measured by flow staining. (E–J) On Day 11, (E) TDLN and spleen IFN\(\gamma\) ELispot were performed with AH1 peptide stimulation. Naive mice served as control. (F and G) Frequency of (F) AH1 tetramer\(^*\) and (G) KIt67\(^*\) of TDLN or splenic CD8\(^+\) T cells. (H) IFN\(\gamma\) production by splenic CD8\(^+\) T cells were tested with CT26 cells stimulation ex vivo. (I) Frequency of AH1\(^+\)KLRG1\(^+\) and AH1\(^+\)KLRG1\(^+\) of splenic CD8\(^+\) T cells. (J) Adoptive transfer of purified CD45.1\(^+\) CD8\(^+\) donor T cells from CT26 tumor-bearing mice into CD45.2\(^+\) mice (recipient, n = 8). Mice were immunized with HBSS, LADD-AH1, and LADD one day later. Seven days postimmunization, frequency of AH1 tetramer\(^*\) of donor and recipient CD8\(^+\) T cells, B–D were analyzed by one-way ANOVA with Tukey’s multiple comparisons test, E–J were analyzed by Mann–Whitney U tests. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001. Results are representative of at least two independent experiments.
infiltrating CD8⁺ T cells (Fig. 3 A and B and SI Appendix, Fig. S5A). Interestingly, PD-1 expression on CD8⁺ T cells decreased with both LADD and LADD-AH1 treatment (Fig. 3C). Remarkably, treatment with LADD-AH1 but not LADD led to a massive increase in IFNγ-producing AH1-specific CD8⁺ T cells in the TME (Fig. 3 D and E). Furthermore, the principal increased population of AH1⁺ CD8⁺ T cells in the TME induced by LADD-AH1 treatment were AH1⁺KLRG1⁺ CD8⁺ effector cells (Fig. 3F and SI Appendix, Fig. S5B). RNA-Seq analysis revealed distinct gene signatures between the AH1⁺KLRG1⁻ and AH1⁺KLRG1⁺ CD8⁺ T cell populations in LADD-AH1-treated tumors (Fig. 3G). AH1⁺KLRG1⁻CD8⁺ T cells expressed higher levels of Gzma, Ifng, and Cd5, but lower levels of Pdcd1, Lag3, Clda4, and Socs3 compared with AH1⁺KLRG1⁺CD8⁺ T cells (Fig. 3G and SI Appendix, Table S2). Flow analysis confirmed that AH1⁺KLRG1⁺CD8⁺ T cells expressed lower levels of PD-1, Lag3 (Fig. 3H and SI Appendix, Fig. S5 C and D) and produced higher levels of IFNγ (Fig. 3I), indicating that LADD-AH1 induced a functional AH1⁺KLRG1⁺ CD8⁺ T cell population that was not exhausted.

To test AH1⁺KLRG1⁺ CD8⁺ T cells primed in the spleen that subsequently trafficked into the TME, we used the sphingosine 1-phosphate analog FTY720, a drug that inhibits egress of lymphocytes including CD8⁺ T cells from secondary lymphoid organs (22). While similar percentages of AH1⁺CD8⁺ T cells were present in the TME of HBSS- or FTY720-treated mice, tumor-infiltrating AH1⁺CD8⁺ T cells were reduced in LADD-AH1-treated mice that also received FTY720 (Fig. 3F). Together, these results indicate that while the LADD itself induced changes in the TME, only LADD-AH1 induced IFNγ-producing AH1⁺KLRG1⁺ effector T cells that traffic to tumors.

TME Remodeling Is Dependent on LADD-Induced Tumor-Specific CD8⁺ T Cells. The TME is highly immunosuppressive partially due to inhibitory cell populations, including Tregs, MDCs, and TAMs. We next characterized these populations in the TME after LADD treatment. LADD-AH1 treatment decreased tumor-infiltrating Treg frequency (Fig. 4A) which combined with the high magnitude of AH1⁺CD8⁺ effector T cells in the TME (Fig. 3 A and B) dramatically increased the ratio of CD8⁺ T cells to Tregs (CD8/Treg) (P < 0.0001; Fig. 4B). Interestingly, LADD treatment also decreased Treg frequency (Fig. 4A) and increased CD8/Treg ratio (P < 0.05) (Fig. 4B), consistent with TME modification by the LADD vector itself.

Interestingly, treatment with LADD-AH1 but not LADD induced iNOS production and prevented CD206 expression by TAMs, and this phenotype was completely abrogated by CD8⁺ T cell depletion (Fig. 4 C and D). These results indicate that the tumoral M2 to M1 macrophage shift depended on LADD-AH1-induced CD8⁺ T cells. TAM reprogramming following Lm phagocytosis has been previously reported (23). This is unlikely given that LADD-AH1 was enriched in the spleen and liver, but not the CT26 tumors of mice (Fig. 4E), and LADD was unable to induce TAM reprogramming. LADD-AH1 was cleared 7 d postinjection, when CT26 tumors started collapsing (SI Appendix, Fig. S6A). The discrepancy between our observation and previous reports may be due to difference in models.

To test whether CD8⁺ T cells could directly mediate TAMs repolarization, we cocultured splenic CD8⁺ T cells with an untreated CT26 tumor suspension ex vivo. Indeed, the presence of LADD-AH1-induced AH1⁺CD8⁺ effector T cells increased iNOS production and decreased CD206 expression in TAMs (Fig. 4 F and G). Increased iNOS production was blocked by α-IFNγ, while CD206 expression was unaffected (Fig. 4 F and G). These data suggested that IFNγ produced by AH1⁺CD8⁺ T cells is essential for iNOS production but not CD206 down-regulation.

To further test the role of TAMs in vivo, we employed anti-CSF-1R and anti-CCL2 antibodies to prevent TAMs recruitment into the tumor (24, 25). Anti-CSF-1R/CCL2 treatment delayed CT26 tumor growth, which might be a result of M2 removal from the TME (SI Appendix, Fig. S6B). Furthermore, LADD-AH1 still controlled CT26 tumor growth following anti-CSF-1R/CCL2 treatment although we observed a tendency toward increased tumor burden in some LADD-AH1–treated mice. These data suggest that TAMs are not essential for LADD-Ag efficacy.

We further examined the TME cytokine and chemokine profile by luxemin. Consistent with TAMs repolarization, inflammatory cytokines including IFNγ and TNFα dramatically increased in the TME upon LADD-AH1 immunization (Fig. 4 H and I), which depended on LADD-AH1–induced CD8⁺ T cells.

![Fig. 3](https://www.pnas.org/cgi/doi/10.1073/pnas.1801910115) Deng et al.
Remodeling of TME is dependent on LADD-AH1 = 0.001, and population. level (picograms per milliliter) in supernatants of tumor. In scatter plots, each for 24 h. (H4) were i.v. injected with LADD-AH1 on day 7. Lm 0.01, **G < level (picograms per milliliter) (T cells from LADD-AH1 < August 7, 2018 | 0.05 and ****10 E5) and survivors treated with control IgG or LADD-AH1 induces memory effector CD8+ T cells that protect survivors from CT26 rechallenge. Survivors and naive mice were rei noculated with CT26 in their left thoracic flanks. (A-D) Before reinoculation, (A) frequency of peripheral AH1 tetramer+ CD8+ T cells, (B-D) Representative staining of AH1 tetramer and (B) KLRG1, (C) Ki67, or (D) T-bet of peripheral CD8+ T cells in survivors. (E) S.c. growth of 5 × 10^6 CT26 in naive mice (n = 5) and survivors treated with control IgG or α-CD8. Tumor volumes ±SEM are shown. In scatter plots, each circle represents one mouse. FACS plots show representative analysis for one mouse per group. A was analyzed by Mann-Whitney U tests. E was analyzed by two-way ANOVA. *P < 0.05 and ****P < 0.0001. Results are representative of at least two independent experiments.
tumor implantation itself primed AH1+CD8+ T cells, which could be amplified by LADD-AH1 immunization (Fig. 2F). These data are consistent with previous observations that AH1 is a weak agonist for its cognate T cell receptor [Kp = 5.7 μM, 1/Δα = 2 (27)] and the dominant target for CT26-specific CTL responses (15). In contrast, LLO responses were comparable in tumor-bearing and naïve mice because LLO is strongly immunogenic and is presented to CD4+ and CD8+ T cells through distinct MHC levels (28).

Tumor growth can proceed unabated despite abundant tumor-specific T cells in the TME (Fig. 3D), indicating that these T cells are dysfunctional. Rapid induction of dysfunctional tumor-specific T cells occurs through continuous antigen encounter in an immunosuppressive TME (2). It is well-established that microbial infection can induce protective pathogen-specific T cells (9). In this study, we utilized attenuated Lm to generate functional cytotoxic CD8+ T effector cells that overcome the immunosuppressive TME and promote tumor rejection. LADD-Ag delivers antigens into the cytoplasm of infected cells such as dendritic cells (DCs) for processing and presentation on MHC molecules, which provides a sustained source of tumor antigen and innate immune stimulation. In this context, tumor-specific T cells were amplified where tumor antigen was presented on activated DCs. The phenotypic CX3CR1+CD62L- KLRG1+PD-1 profile of LADD-Ag-induced tumor-specific CD8+ T cells resembled that of resident tumor-specific effector cells (11, 29). The phenotype of LADD-Ag induced CD8+ T cells allowed us to distinguish them from resident tumor-specific T cells. Transcriptome analysis further revealed distinct functional signatures of tumor-specific T cells resulting from LADD-based immunotherapy. Tumor-specific T cells induced in the inflammatory context were less exhausted, produced IFNγ, controlled tumor growth, and established long-term memory.

Both antigens and adjuvants remain the focus of questions surrounding the optimal design of cancer vaccines. Viral antigens and neoantigens are considered ideal targets because of the lack of central tolerance (8). The preclinical potency of LADD expressing the viral antigens AH1 and E7 highlights the therapeutic potential of targeting viral antigens. Indeed, numerous therapeutic vaccination strategies against E7 have been developed for the treatment of cervical cancer and HPV-associated disease (30). Moreover, the strength of vaccine-induced T cell immune responses correlates with the clinical response (31). Interestingly, human endogenous retroviral antigens are emerging as novel immunotherapy targets (32). Notably, gp70 levels are strikingly high in murine tumor cell lines (16), and the gp70-derived AH1 epitope is highly immunodominant, which could explain both LADD-AH1 antitumor efficacy and the lack of epitope spreading against other known CT26 antigens. In addition to viral antigens, LADD-Neo can elicit neoantigen-specific CD8+ T cells and TME changes in a preclinical mouse model, which provides rationale for LADD treatment targeting neoantigens. We recently initiated a clinical trial targeting neoantigens in patients with microsatellite stable colorectal cancer (NCT03189030). The possibility of developing antigen-loss variants is limited by designing LADD expressing multiple neopeptides that can activate T cells against a variety of antigens. Many platforms are currently used for cancer vaccine development. Compared with the other platforms we tested, LADD appears to be an optimal vehicle for active immunotherapy in mice. Further experiments are required to fully understand its superior activity in preclinical models.

Materials and Methods

LADD-Ag strains, cells, antibodies, and other reagents used in this study are described in SI Appendix. Detailed information on immunization, in vivo tumor experiments, antibody staining and flow cytometry analysis, IFNγ ELISPOT and ICS assays, isolation of CD8+ T cells and RNA-Seq, adoptive transfer of CD8+ T cells, luminescence, IHC staining and histological analysis of CD8 infiltration, LADD biodistribution studies, and statistics can be found in SI Appendix. This study was performed according to protocols approved by the Institutional Animal Use Committee of Aduro Biotech.

Acknowledgments.

We thank Weiquin Liu, Yusup Chang, and Bruna Hedberg for support of the study; Hector Nolla and Alma Valeros at University of California, Berkeley for help with cell sorting; and Leticia Castaneda for preparing the Graphical Abstract. We are grateful to David Raulet and Erin Benanti for helpful comments. We thank the research department at Aduro Biotech for useful discussion and comments on the manuscript.

1. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A (2017) Primary, adaptive, and acquired resistance to cancer immunotherapy. Cell 168:707–723.
2. Schietinger A, et al. (2016) Tumor-specific T cell dysfunction is a dynamic antigen-driven process that can be initiated early during immunogenesis. Immunity 43:389–401.
3. Channey T, Ontong P, Konno K, Iano N (2014) Tumor-associated macrophages as major players in the tumor microenvironment. Cancers ( Basel ) 6:1670–1690.
4. Stein M, Keshav S, Harris N, Gordon S (1992) Interleukin 4 potently enhances murine macrophage mannose receptor activity: A marker of alternative immunologic macrophage activation. J Exp Med 167:287–292.
5. Dalton DK, et al. (1993) Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. Science 259:1710–1712.
6. Khalil DN, Smith EL, Brentjens RJ, Wolchok JD (2016) The future of cancer treatment: Immuno modulation, CARs and combination immunotherapy. Nat Rev Clin Oncol 13:273–290.
7. Caffman RL, Sher A, Seder RA (2010) Vaccine adjuvants: Putting innate immunity to work. Immunity 33:492–503.
8. Meiler CJ, van Hall T, Arens R, van der Brug SH (2015) Therapeutic Cancer vaccines. J Clin Invest 125:3401–3412.
9. Harly JT, Tvinneire AR, White DW (2000) CD8+ T cell effector mechanisms in resistance to infection. Annu Rev Immunol 18:275–308.
10. McMahon CW, et al. (2002) Viral and bacterial infections induce expression of multiple NK cell receptors in responding CD8+ T cells. J Immunol 169:1452–1457.
11. Olson JA, McDonald-Hymann C, Jameson SC, Hamilton SE (2013) Effector-like CD8+ T cells in the memory population mediate potent protective immunity. Immunity 38:1250–1260.
12. Glemski li, Descart AL, Portney DA (2003) Listeria monocytogenes mutants that fail to compartmentalize listerolysin O activity are cytotoxic, avirulent, and unable to evade host extracellular defenses. Infect Immun 71:6754–6765.
13. Cosart P, Pezaro-Cerda J, Lecuit M (2003) Invasion of mammalian cells by Listeria monocytogenes: Functional mimicry to subvert cellular functions. Trends Cell Biol 13:33–37.
14. Brockstedt DG, et al. (2004) Listeria-based cancer vaccines that segregate immunogenicity from toxicity. Proc Natl Acad Sci USA 101:13832–13837.
15. Huang AY, et al. (1996) The immunodominant major histocompatibility complex class I-restricted antigen of a murine colon tumor derives from an endogenous retroviral product. Proc Natl Acad Sci USA 93:9730–9735.
16. Scrimieri F, et al. (2013) Murine leukemia virus envelope gp70 is a shared biomarker for the high-sensitivity quantification of murine tumor burden. Oncol Immunol 2: e26889.