Intracellular Bacterial Vectors That Induce CD8+ T Cells with Similar Cytolytic Abilities but Disparate Memory Phenotypes Provide Contrasting Tumor Protection

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Abstract

Induction of a functional CD8+ T-cell response is the important criterion for cancer vaccines, and it is unclear whether acute or chronic live vectors are better suited for cancer antigen delivery. We have evaluated the tumor protective ability of two recombinant vectors, Listeria monocytogenes (LM) and Salmonella typhimurium (ST), both expressing ovalbumin (OVA). Although both vectors induced a similar OVA-specific CD8+ T-cell response in the long term, LM-OVA induced mainly central-phenotype (T_CEM: CD44highCD62Lhigh), whereas ST-OVA induced mainly effector-phenotype (T_EM: CD44highCD62Llow) cells. Both vectors induced functional OVA-specific CD8+ T cells that expressed IFN-γ and killed targets specifically in vivo. However, only LM-OVA–vaccinated mice were protected against B16-OVA tumors. This correlated to the ability of CD8+ T cells generated against LM-OVA, but not against ST-OVA, to produce interleukin 2 and exhibit profound homeostatic and antigen-induced proliferation in vivo. Furthermore, adoptive transfer of memory CD8+ T cells generated against LM-OVA (but not against ST-OVA) into recipient mice resulted in their trafficking to tumor-draining lymph nodes conferring protection. Although cytotoxicity and IFN-γ production are considered to be the principal functions of memory CD8+ T cells, the vaccine delivery strategy may also influence memory CD8+ T-cell quality, and ability to proliferate and traffic to tumors. Thus, for efficacy, cancer vaccines should be selected for their ability to induce self-renewing memory CD8+ T cells (CD44highCD62Lhigh) besides their effector functions.

Introduction

A major goal of cancer immunotherapy is to generate long-lived CD8+ T cells for efficacious protection. Despite the success in generating CD8+ T-cell responses to self antigens (1–6), cancer immunotherapy has provided objective long-term remission in only a small proportion of patients with select cancer types (7). Moreover, cancer resurgence occurs despite the presence of tumor-specific CD8+ T cells (8).

Memory CD8+ T cells are heterogeneous in phenotype: effector-memory (T_EM) and central-memory (T_CEM) cells (9–11). Although both subtypes are antigen experienced, T_EM have a decreased expression of CD62L that allows them to take up residence in peripheral nonlymphoid tissues, whereas T_CEM are CD62Lhigh allowing them to localize to lymphatic tissues, and proliferate profoundly when rechallenged with antigen (9, 12–14). Thus, T_CEM are considered to be more potent at mediating protection against pathogens (13, 15–17).

Studies with viral infections have shown that the CD8+ T-cell response peaks at day 7, which is followed by a massive contraction when ~90% of primed cells perish (18). However, because varied pathogens have distinct replication rates, intracellular/extracellular habitations and differ in types of inflammation-induced, one unified model of CD8+ T-cell priming does not accurately describe the pattern of priming and memory induction against all types of pathogens, particularly those leading to chronic infection (19–21). Given the heterogeneity of CD8+ T-cell responses, it is unclear what kind of delivery system should be selected to promote an effective CD8+ T-cell response against tumors.

We have used two bacterial vectors, Listeria monocytogenes (LM) and Salmonella typhimurium (ST), as antigen delivery systems and addressed how the qualitative differences in CD8+ T-cell responses may influence protection against tumors. We show that long-term antitumor immunity against B16-ovalbumin (OVA) melanoma is more efficacious after delivery of OVA by LM rather than ST. This correlates to the distinct phenotypes of memory cells despite functional cytolytic CD8+ T cells induced by the two vectors.

Materials and Methods

Bacterial strains and assessment of bacterial burden. Recombinant ST and LM expressing the gene for OVA (ST-OVA and LM-OVA) were generated and grown as described previously (20, 22). Organs of infected mice were homogenized in 0.9% NaCl and colony-forming units (CFU) were determined by plating 100 µL of serial 10-fold dilutions on BHI agar plates (Difco laboratories).

Mice and immunizations. C57BL/6, 129x1/SvJ, B6.129S7-Kitl<+Msd>/J (lacking mature lymphocytes), and OT.1 TCR transgenic mice (with CD8+ T cells expressing the OVA257-264 TCR) were obtained from The Jackson Laboratory. B6.129F1 mice were generated by mating 129x1/SvJ females with C57BL/6 males; they were able to survive an ST-OVA infection and receive transgenic OT.1 cells or B16-OVA cells that are both of C57BL/6 origin. Mice were maintained in accordance with the guidelines of the Canadian Council on Animal Care. For immunization, frozen stocks of B16-OVA (expressing the gene for OVA) cells were obtained from Dr. Edith Lord (University of Rochester, Rochester, NY) and cultured as described previously (22). Solid tumors were induced with s.c. injection of 1 × 106 B16-OVA cells. Tumors were inoculated with 1 × 106 B16-OVA cells. Tumors in B6.129F1 mice were
pooled spleen cells of infected mice using the CELLection Biotin Binder with antibodies against CD8, CD62L, IL-7R.

Assessment of the fate and phenotype of antigen-specific CD8+ T cells. At various time intervals after infection, spleen or blood cells were stained with antibodies against CD8, CD62L, IL-7R, CD44, and H-2KbOVA257-264 tetramer (20). All antibodies were obtained from BD Biosciences. H-2KbOVA257-264 tetramer was obtained from Beckman Coulter. Cells were washed with PBS, fixed in 0.5% formaldehyde, and acquired on BD Biosciences fluorescence-activated cell sorting Canto analyzer.

Dendritic cell purification and antigen presentation to OT.1 CD8+ T cells. Individual spleens were homogenized with 400 μL of 10 mg/mL collagenase in RPMI for 30 min at 37°C. T cells and B cells were depleted with anti-Thy1.2 and anti-B220, and dendritic cells were positively selected with anti-CD11c antibodies using Miltenyi Biotech magnetic separation. CFUs were enumerated for the purified dendritic cell fraction. To assess antigen presentation, dendritic cells (10^5/well) were cocultured for 4 d with CFSE-stained OT.1 splenocytes (10^5/well), in 96-well microtiter plates, in RPMI containing 8% fetal bovine serum (FBS) and 50 μg/mL gentamicin. Positive controls included cells cultured with 5 μg/mL of OVA257-264 peptide, whereas negative controls included OT.1 cells cultured without dendritic cells. Proliferation of OVA-specific CD8+ T cells was measured 4 d later by assessing CFSE reduction.

Assessment of homeostatic proliferation, trafficking, and protection by memory CD8+ T cells. For in vitro homeostatic proliferation, 125 to 146 d postinfection splenocytes were stained with CFSE (0.125 μmol/L) and cultured (10 × 10^6 cells/ml in R8 medium) with IL-7 (10 ng/mL) and IL-15 (10 ng/mL). At days 1 to 11, culture aliquots were stained with anti-CD8 antibody and CD8+ OVA257-264-tetramer, and carboxyfluorescein diacetate succinimidyl ester (CFSE) loss was monitored to assess cell division. For in vivo evaluation of homeostatic proliferation, 91 d postinfection, splenocytes were stained with CFSE, CD8+ T cells purified by negative selection and injected into Rag2-/- mice (3 × 10^6 cells per mouse). Seven days later, CFSE loss (proliferation) in adoptively transferred antigen-specific cells of recipients was evaluated. For assessment of trafficking, CFSE-labeled CD8+ T cells (purified by negative selection) were transferred (15 × 10^6/mouse, i.v.) into naive B6129F1 mice. Seven days later, some recipients were challenged with 10^6 B16-OVA cells (s.c.). Three days posttumor challenge (10 d postadoptive cell transfer), the numbers of CFSE-labeled antigen-specific donor cells in organs of recipients were enumerated by flow cytometry. Tumor-draining (inguinal and popliteal) lymph nodes were pooled for analysis. To confirm tumor protection by CD8+ T cells, purified (positive selection) memory cells (4 × 10^6/mouse, i.v.) were transferred to B6129F1 mice, which were then challenged with B16-OVA (s.c.).

Results

LM-OVA induces a better tumor protection compared with ST-OVA. Protection against s.c. B16-OVA tumor was tested after prophylactic exposure to LM-OVA and ST-OVA. Naïve or OT.1-parkel control nonvaccinated mice rapidly developed large tumors, succumbing by 3 weeks. In contrast, 90 to 92 days postinfection, only 20% of LM-OVA vaccinated mice developed tumors, whereas 50% of ST-OVA mice developed tumors (Fig. 1A). Based on 3 independent experiments wherein mice were challenged 51 to 136 days postinfection, nonvaccinated mice had palpable day 5 onwards and were expressed in mm^2 (obtained by multiplication of perpendicular measurements).

Assessment of in vivo homeostatic proliferation, trafficking, and protection by memory CD8+ T cells. For in vitro homeostatic proliferation, 125 to 146 d postinfection splenocytes were stained with CFSE (0.125 μmol/L) and cultured (10 × 10^6 cells/ml in R8 medium) with IL-7 (10 ng/mL) and IL-15 (10 ng/mL). At days 1 to 11, culture aliquots were stained with anti-CD8 antibody and CD8+ OVA257-264-tetramer, and carboxyfluorescein diacetate succinimidyl ester (CFSE) loss was monitored to assess cell division. For in vivo evaluation of homeostatic proliferation, 91 d postinfection, splenocytes were stained with CFSE, CD8+ T cells purified by negative selection and injected into Rag2-/- mice (3 × 10^6 cells per mouse). Seven days later, CFSE loss (proliferation) in adoptively transferred antigen-specific cells of recipients was evaluated. For assessment of trafficking, CFSE-labeled CD8+ T cells (purified by negative selection) were transferred (15 × 10^6/mouse, i.v.) into naive B6129F1 mice. Seven days later, some recipients were challenged with 10^6 B16-OVA cells (s.c.). Three days posttumor challenge (10 d postadoptive cell transfer), the numbers of CFSE-labeled antigen-specific donor cells in organs of recipients were enumerated by flow cytometry. Tumor-draining (inguinal and popliteal) lymph nodes were pooled for analysis. To confirm tumor protection by CD8+ T cells, purified (positive selection) memory cells (4 × 10^6/mouse, i.v.) were transferred to B6129F1 mice, which were then challenged with B16-OVA (s.c.).
a median survival of ~20 days, and ST-OVA–vaccinated mice exhibited a median survival of 41 days. In contrast, the majority of LM-OVA–vaccinated mice survived beyond 100 days (Fig. 1B). In these experiments, 10^5 OT-1 CD8+ T cells were transferred before vaccination, to enable long-term tracking of antigen-specific responses. However, even in the absence of OT.1 transfer, divergence in tumor protection between LM-OVA and ST-OVA was observed (Supplementary Data). Tumor growth in mice injected with nonrecombinant LM or ST was similar to uninfected mice (data not shown), confirming lack of bystander vector effects. Therefore LM-OVA and ST-OVA clearly afforded contrasting long-term antigen-specific tumor protection.

**Figure 2.** Kinetics of bacterial burden and CD8+ T-cell response. B6129F1 mice were infected with 10^3 LM-OVA or ST-OVA. A, mean CFU ± SE (n = 2–4 per time point per group). Detection limits were 100 CFU (dotted line) except for lung tissue wherein the detection limit was 1 CFU. B, CFU (left) were enumerated in the spleens and purified dendritic cells of individual mice (n = 3 per time point). Antigen presentation based on % CFSE loss (right) in OT.1 CD8+ splenocytes cultured for 4 d with purified dendritic cells (obtained from 3 individual mice per time point). C, B6129F1 mice were injected with 10^5 OT.1 CD8+ T cells and 10^3 LM-OVA or ST-OVA. Representative flow-cytometric profile indicating percentage of OVA-specific CD8+ T cells and kinetics of response indicating their numbers (points, mean of n = 2 mice per group per time point; bars, SE). D, OVA257-264–specific CD8+ T cells pretumor and posttumor challenge. n = 3 to 5 mice per group per time point. For posttumor time points in the ST-OVA group, data are representative of surviving mice only. Tumor challenge was done 62 d (top) or 90 to 92 d (bottom) postinfection. After tumor challenge, the number of antigen-specific CD8+ T cells in the LM-OVA group increased significantly (P < 0.05 by paired t test). All data are representative of two to three separate experiments conducted.

LM-OVA and ST-OVA induce differential infection pattern and CD8+ T-cell kinetics. LM-OVA infection peaked early and was undetectable after 7 days in the various organs, whereas ST-OVA showed a delayed peak. Nevertheless, after ST-OVA infection, bacterial numbers dramatically decreased by 60 days in all organs tested and were undetectable beyond 100 days (Fig. 2A). Furthermore, LM-OVA were present in purified dendritic cell fraction on day 1 after infection but not detectable at day 15 (Fig. 2B, left). In contrast, ST-OVA were absent from dendritic cells on day 1 after infection, and were detected on day 15 (Fig. 2B, left). Accordingly, antigen presentation by LM-OVA dendritic cells (based on OT.1 proliferation) occurred as early as...
day 1 after infection. In contrast, the delayed uptake of ST-OVA by dendritic cells corresponded to delayed antigen presentation (Fig. 2B, right).

We then measured the kinetics of the CD8+ T-cell response. LM-OVA-induced splenic OVA257-264 CD8+ T cells peaked in number by day 7; this was followed by a massive contraction resulting in 10-fold reduction in numbers (Fig. 2C). Alternatively, ST-OVA induced delayed CD8+ T-cell response peaking at day 21 followed by a prolonged contraction phase (Fig. 2C). Thus, LM-OVA evoked an acute infection and rapid CD8+ T-cell response, whereas ST-OVA evoked a chronic infection characterized by delayed dendritic cell uptake, antigen presentation, and CD8+ T-cell response.

**Tumor challenge expands memory CD8+ T cells in LM-OVA but not ST-OVA vaccinated mice.** Before tumor challenge, at 60 days and beyond of infection, the number of memory cells in the blood were low (3–6%) but similar in LM-OVA and ST-OVA–vaccinated mice (Fig. 2D). In LM-OVA–vaccinated mice, tumor challenge at day 62 or 92 caused a rapid expansion of OVA CD8+ T cells (to ~10–12%). In contrast, no such increase was observed in ST-OVA–vaccinated mice. Moreover, the numbers of OVA-specific CD8+ T cells in ST-OVA group remained constant even 100 days posttumor ruling out a delayed secondary response.

**LM-OVA memory CD8+ T cells differentiate to a predominant CD62Lhigh phenotype.** The phenotype of CD8+ T cells was characterized by assessing expression of CD44 (activation marker), CD62L, and IL-7Rx (expressed at high levels on naïve and central memory cells). A classic differentiation pattern of CD8+ T cells was observed during LM-OVA infection; an immediate effector phenotype followed by a progressive increase in central memory (CD62Lhigh) cells (Fig. 3A). In contrast, at 3 weeks of ST-OVA infection, a predominant effector phenotype (CD44highCD62Llow) was seen (Fig. 3A). The expression of CD62L on ST-OVA–induced CD8+ T cells crept up from 15% to 30% by day 60 in the spleen; however, this was not comparable with the high levels (~70%) observed for LM-OVA. IL-7Rx expression, which is classically up-regulated on naïve and memory T cells (18) dropped as expected at the respective peak of priming in both infections. Subsequently, a majority of LM-OVA–specific CD8+ T cells seemed to take on the memory phenotype (IL-7Rxhigh) as early as 22 days, and ~80% of the cells were IL-7Rxhigh thereon (Fig. 3B). In contrast, peak reduction in IL-7Rx expression occurred only after 3 weeks of ST-OVA infection correlating with the delay in priming. Subsequently, IL-7Rx expression gradually increased over time (Fig. 3B). Comparing the relative numbers of CD8+ TCM cells (CD62LhighIL-7Rxhigh), by day 76, LM-OVA group exhibited around 86%, whereas

![Figure 3.](https://www.aacrjournals.org/can/2009/69/10/fig3.jpg)

**Figure 3.** Expression of memory markers on CD8+ T cells. The expression of CD62L (A) and IL-7Rx (B) gated on CD44+ OVA–specific CD8+ T cells in mice that received 105 OT.1 CD8+ T cells and 103 LM-OVA or ST-OVA. Data are based on expression levels in n = 2 to 10 per group per time point; points, mean; bars, SE. C, representative plot demonstrating coexpression of CD62L and IL-7Rx on gated CD44+, OVA–specific CD8+ T cells in the blood. Numbers indicate % within each quadrant. Data are representative of two separate experiments conducted. ***, significant difference (P < 0.001 by two-way ANOVA) between treatment groups.
ST-OVA-induced memory CD8\(^+\) T cells are fully functional. Next, we determined whether LM-OVA and ST-OVA infection evoke disparity in CD8\(^+\) T-cell function. The in vivo cytotoxicity assay measures the killing ability of CD8\(^+\) T-cells in an immediate 24-hour time period. Seven days postinfection, 99.8% of target cells were eliminated specifically in LM-OVA–infected mice, whereas the peak of cytolytic activity in ST-OVA–infected mice was \(~ 86\%\) but not observed until 30 to 60 days after infection (Fig. 4A). However, CD8\(^+\) T-cell cytolytic ability was low (\(~ 20\%)\) in LM-OVA–infected mice by day 14 (Fig. 4A), which corresponded with clearance of infection and contraction of the response. Noticeably, ST-OVA CD8\(^+\) T cells maintained strong immediate cytolytic functions up to 60 days (Fig. 4A).

OVA-specific CD8\(^+\) T cells induced against LM-OVA and ST-OVA expressed similar levels of IFN-\(\gamma\) at all time points tested (Fig. 4B). In contrast, only LM-OVA CD8\(^+\) T cells expressed increased intracellular IL-2, and remarkably, their numbers were stably maintained over prolonged periods (Fig. 4C). ST-OVA CD8\(^+\) T cells showed reduced IL-2 expression at all times (Fig. 4C). Thus, it seems that although CD8\(^+\) T cells generated against ST-OVA are fully functional in their ability to kill targets and produce IFN-\(\gamma\), they are unable to produce IL-2, to support their proliferation and expansion.

To rule out that the weak tumor protective ability of ST-OVA CD8\(^+\) T cells was not related to the presence of T regulatory cells, splenic CD4\(^+\)CD25\(^{\text{high}}\)FoxP3\(^{+}\) cell numbers were determined. For both infections, splenic T regulatory cell numbers remained similar over time (Fig. 4D).

ST-OVA–generated memory CD8\(^+\) T cells exhibit defective homeostatic proliferation and trafficking to lymphoid organs. Homeostatic proliferation by antigen-specific CD8\(^+\) T cells can facilitate their continued survival in the wake of an aggressive tumor. CD8\(^+\) T cells generated after LM-OVA infection were able to homeostatically proliferate (based on CFSE reduction) in the presence of growth cytokines for significantly prolonged period compared with those generated after ST-OVA infection (Fig. 5A). CD8\(^+\) T cells died rapidly in culture in the absence of cytokine supplementation (Fig. 5A). In vivo, transfer of day 90 OVA-specific CD8\(^+\) T cells into naïve RAG\(^{-/-}\) recipients resulted in significantly higher proliferation for LM-OVA memory cells compared with ST-OVA (Fig. 5B). Because RAG\(^{-/-}\) mice lacked endogenous T cells, adoptively transferred memory cells proliferated homeostatically. Next, we tracked the ability of adoptively transferred memory CD8\(^+\) T cells to traffic efficiently in response to tumor challenge in B6129F1 mice. In this scenario, greater numbers of LM-OVA (but not ST-OVA) memory CD8\(^+\) T cells trafficked to lymphoid compartments 3 days after tumor challenge (Fig. 5C) relative to nontumor-bearing recipients. Thus, ST-OVA CD8\(^+\) T cells were qualitatively defective in homeostatic expansion ability and homing capacity.

Adoptively transferred LM-OVA CD8\(^+\) T cells exhibit superior tumor protection. Infections also cause the activation of antigen presenting cells (APCs) and inflammation that may provide bystander tumor protection. Therefore, ST-OVA and LM-OVA memory CD8\(^+\) T cells were purified 71 days postinfection, and similar numbers of OVA-specific cells were injected into naïve recipients. Seven days later, recipients were challenged with B16-OVA cells (Fig. 6). OVA-specific CD8\(^+\) T cells in the blood of recipients before tumor challenge was <0.1% (data not shown). A significant increase was observed 9 days posttumor injection only...
in recipients that received memory CD8+ T cells from LM-OVA-infected mice (Fig. 6A and B). Accordingly, mice that received LM-OVA memory cells survived 42 days compared with those given ST-OVA CD8+ T cells that succumbed to tumors by 15 to 18 days. As the adoptively transferred CD8+ T cells harbored no bacteria (confirmed by plating purified cells), an inherent defect of ST-OVA-generated CD8+ T cells was apparent rather than effects of inflammation or bacterial persistence.

Discussion

Tumor vaccine delivery approaches aimed at inducing CD8+ T-cell responses have largely focused on maximizing the magnitude of the response to improve efficacy. We show that the phenotype of CD8+ T-cell response to the same antigen may be differentially skewed by immunization strategy. Thus, adjuvants need to be selected for their capacity to influence the appropriate "quality" of memory CD8+ T cells rather than solely for their potential to induce cytotoxic cells expressing IFN-γ.

We have previously reported that many factors such as the timing and extent of antigen-presentation and inflammation influences CD8+ T-cell differentiation (19, 20, 23). Whereas LM-OVA induces a typical T-cell memory paradigm (25) first observed in viral infection models (26), ST-OVA induces a delayed and reduced peak of CD8+ T-cell response followed by a protracted contraction phase perhaps due to its residence within the phagosomes of infected cells (20, 27). As both LM-OVA and ST-OVA express similar levels of OVA (20), clearly, the kinetics of response was influenced by vector properties rather than antigen expression levels. Indeed, the delayed priming of CD8+ T cells by ST-OVA may be correlated to its delayed uptake by dendritic cells. Nevertheless, despite the delay in antigen-presentation by ST-OVA, CD8+ T cell activation does occur albeit at initial lower levels compared with LM-OVA. However, 60 days after infection with either pathogen a stable population (3–6%) of OVA257-265, CD8+ T cells was detected in the blood, and similar numbers (∼2 × 105 cells) were discernable in the spleen. Furthermore, at later times, a majority of the ST-OVA CD8+ T cells had up-regulated IL-7Ra expression that correlates to memory transition (28). Therefore, even with a chronic vector such as ST, a priming phase followed by transition to memory occurred. Thus, tumor challenge was intentionally carried out 60 days and beyond, when similar numbers of memory cells were present in both infections.

Although it was initially thought that TLM cells are better suited to mediate protection, it was shown that TCM cells, due to their ability to proliferate mediated better protection against Lymphocytic choriomeningitis virus (13). Similarly, in vitro generated TCM compared with TEM cells in combination with a tumor antigen vaccine were better able to abolish an established tumor (29). Furthermore, clinical trials involving adoptive T-cell transfer of in vitro expanded tumor-infiltrating lymphocytes to lymphodepleted cancer patients yield ∼50% response rates (30). Therefore, if the appropriate phenotype of CD8+ T cells is promoted, efficient cancer elimination may follow.
Recently, the conditions necessary for the generation of a predominant T_{CM} memory responses have begun to be uncovered (31–33). We showed previously that weak antigen presentation as afforded by Mycobacterium bovis BCG-OVA promotes differentiation mainly into T_{CM} phenotype (23). Furthermore, competition of CD8+ T cells for access to APCs also influences the phenotype distribution (23, 32). BCG-OVA and ST-OVA are both phagosomal resident pathogens that evoke delayed antigen presentation. However, BCG vectors cause T_{CM} generation and afford strong tumor protection (22), suggesting that chronicity itself may not be a detriment for cancer vaccine delivery. Overall, several aspects of ST-OVA infection such as prolonged inflammation, rapid rate of replication (20), delayed uptake by dendritic cells, and delayed clearance likely contribute to continued low-level antigen presentation disallowing differentiation to T_{CM} phenotype.

Chronic antigen exposure has been associated with an eventual ineffective immune response against viral infections (34–36). Chronically stimulated T cells can also become senescent in a tumor-bearing host (8). However, CD8+ T cells induced against ST-OVA were not anergized, or nonfunctional because they were cytotoxic and expressed IFN-γ rapidly. The drop in the in vivo cytolytic activity in LM-OVA–infected mice from day 7 to day 30 is commensurate with the contraction of the antigen-specific CD8+ T-cell response that occurs after priming. Similarly, the delayed and prolonged effector and effector-memory phase of OVA-specific CD8+ T-cell response during ST-OVA infection correlates with the kinetics of in vivo cytotoxicity data. However, at day 60 after infection, although similar numbers of OVA-specific CD8+ T cells were present in LM-OVA and ST-OVA–infected mice, the latter group showed greater in vivo cytolytic activity compared with the former. Effector-memory cells have been shown to exhibit stronger immediate effector function (9), although this remains controversial (37). Nevertheless, our data show how despite a potent immediate cytolytic CD8+ T-cell response, long-term tumor efficacy may be compromised.

The dichotomous ability of ST-OVA and LM-OVA generated CD8+ T cells to protect against tumors correlates to their differential proliferation and capacity to traffic to the tumor site. First, LM-OVA–induced CD8+ T cells are better able to produce IL-2 compared with ST-OVA. Additionally, memory CD8+ T cells characterized persist with homeostatic cytokines IL-7 and IL-15 independently of antigen (38–40). In accordance, only LM-OVA–induced CD8+ T memory cells were able to proliferate enormously in the absence of antigen. Similarly, in response to tumor challenge, the expansion of memory CD8+ T cells occurred only in LM-OVA–vaccinated mice. Although this expansion was small, it was significant because tumors lack the danger signals that activate APC costimulation and cytokine production. Additionally, CD8+ T_{CM} cells evoked by LM-OVA were better able to home to lymphoid organs including tumor-draining lymph node as early as 3 days after tumor challenge. Thus, key qualities of LM-OVA CD8+ T_{CM} cells confer advantages for counteracting the aggressively growing tumor.

Virulent and attenuated strains of ST have been investigated as live vectors for cancer therapy (41, 42). Although such studies reveal induction of CD8+ T cells, their long-term protective ability have not been characterized in detail. Furthermore, ST can elicit profound inflammation and may lead to bystander tumor protection and has been used for its direct oncolytic properties (43, 44). Attenuated ST may also differentially skew T-cell responses (27). LM is also considered an efficient vector for cancer vaccines (45–47). Although we have used highly virulent vectors that may not be desirable for human vaccines, they were intentionally chosen to consistently drive extreme phenotypes of CD8+ T-cell response, T_{CM} and T_{EM}. Thus, they constituted convenient models for critically delineating the factors that may influence T-cell quality and consequent vaccine efficacy.

One consideration is that a 10-fold reduction in pathogen burden by memory T cells may be sufficient to provide complete protection against infection when complemented with the ability of the innate immunity to eliminate the remaining pathogens. However, a meager 10-fold reduction in tumor burden by memory T cells is insufficient in cancer immunotherapy, as tumor cells are not associated with
danger signals, and consequently the innate immune system remains ignorant and unable to complement adaptive immunity. Thus, although TEM cells alone may be sufficient to facilitate protection against pathogens, they may be incapacitated when it comes to tumor control. 

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**References**


**Acknowledgments**

Received 8/14/08; revised 2/16/09; accepted 3/5/09; published OnlineFirst 5/12/09.

Grant support: Ontario Cancer Research Institute and the National Research Council of Canada.

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We thank Komal Gurnani, Ahmed Zafer, and Reni Dudani for providing technical assistance. F. Stark is a recipient of Ontario Graduate Student Science and Technology Scholarship.
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Cancer Res  Published OnlineFirst May 12, 2009.

Updated version  Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-08-3160

Supplementary Material  Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2009/04/27/0008-5472.CAN-08-3160.DC1

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