Introduction

Adoptive T-cell therapy represents a means to treat viral infections, especially in immunocompromised hosts, and to induce tumor regression (1–4). Early work in mouse models indicate that adoptively transferred CD8+ T cells were the optimal lymphocyte population for effective immunotherapy (5, 6). Nevertheless, the transferred CTLs often display transient lytic function and do not persist long term (7–9). Thus, there remains much interest to optimize the conditions used to activate and expand antigen-specific CD8+ T cells ex vivo before infusing them into virally infected or tumor-bearing recipients.

Several important considerations in generating CTLs for adoptive therapy include the priming conditions to select T cells with the optimal T-cell receptor (TCR) affinity, the choice of T-cell growth factors, and the extent of in vitro expansion. Interleukin-2 (IL-2) has been used most often to expand CTLs in vitro, as this cytokine is the most potent T-cell growth factor. However, CTLs that have undergone considerable expansion to interleukin 2 (IL-2) do not persist effectively upon adoptive transfer (10–12). Somewhat paradoxically, early effector cells from 1-week cultures in IL-2 were much more effective in eradication of established tumors than fully differentiated CTLs derived by prolonged culture when transferred to lymphoid-depleted tumor-bearing mice (13). There is mounting evidence that IL-15 may be a superior growth factor for adoptive T-cell therapy. CTLs derived with IL-15 persist long term upon transfer to normal mice and develop antitumor activity (10, 12, 14, 15). Application of IL-15 has also been shown to potentiate antitumor CTL responses in vivo (16).

There are a number of reasons to hypothesize that culture conditions that lead to CTLs that acquire properties of memory cells either in vitro or upon adoptive transfer might exhibit superior efficacy in adoptive T-cell therapy. For example, memory T cells respond to lower doses of antigen, require less costimulatory signals, exhibit more rapid and robust effector function, and their long-term persistence characterizes a sterilizing immune response (17–21). In this regard, we showed that in vitro derived OVA-specific CD8+ OT-I TCR transgenic CTLs acquired many phenotypic and functional properties of memory cells when further cultured in IL-15, but without antigen (10). Upon adoptive transfer of the IL-15-expanded T cells into lymphoid-replete mice, these cells engrafted and persisted long term with properties resembling central memory cells. It remains unclear whether IL-15 was essential for the generation of this persistent cell population or whether it simply reflected the removal of antigen. Even more striking, the short-term adoptively transferred OT-I CTLs readily persisted, uniformly expressed properties of central memory cells, and rejected a tumor challenge (10, 22). Given this efficacy without conditioning the recipients, the current study was undertaken to largely focus on the initial steps as short-term CTL transit to memory-like cells, including the extent that the presence of antigen affected this process, and the ability of the persistent T cells to reject an established solid tumor.

Materials and Methods

Mice. C57BL/6 mice and the human Bcl-2 transgenic [C57BL/6-TgN(Bcl2)36Wehi] mice on the C57BL/6 background were obtained from The Jackson Laboratory. B6SJL-Ptprc/BoAitac mice, congenic for CD45 and expressing the CD45.1 allele, and OT-I (RAG1−/−) mice were obtained from Taconic Farms. OT-I mice were crossed to Bcl-2 transgenic mice to yield OT-I×Bcl-2 mice. These latter mice were RAG1−/− or RAG1+/−.

Cell isolation and culture conditions. The lymphocytes from the liver and lungs were isolated as previously described (23). Spleen cells from the indicated OT-I mice (1 × 106 per well) were cultured in 24-well plates in 1 mL of complete RPMI 1640 (Mediatech, Inc.), with OVA257-264 peptide (0.1 nmol/mL; synthesized by Research Genetics) and mouse IL-2 (10 ng/mL; Peprotech, Inc.) as previously described (10). After 3 d in culture (referred to as day 3 in Results), the cells were harvested, washed thrice with RPMI 1640, and sometimes were recultured in T25 culture flasks at 105 cells/mL in 10 mL of complete medium without OVA257-264, but with IL-2 or IL-15 (10 ng/mL) for 2 additional days (referred to as day 5 in Results). Viability was determined at various time points by trypan blue exclusion or flow cytometry.

Adoptive transfer studies. In vitro generated effector cells were washed thrice in HBSS and resuspended in HBSS. Unless otherwise

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indicated, $1 \times 10^7$ donor cells (0.5 mL) were injected i.v. into recipient mice. To measure 5-bromo-2-deoxyuridine (BrdUrd) incorporation, recipient mice received drinking water containing 0.8 mg/mL of BrdUrd (BD PharMingen) for 3 d starting at the day of the adoptive transfer. In some studies, CD45.1 congenic C57BL/6 recipient mice received E.G7 tumor cells s.c. in the abdomen. The tumor developed for 10 to 14 d until a palpable tumor was established at 50 mm$^3$. At this time, the tumor-bearing recipients were adoptively transferred with OT-I effector cells or left untreated.

**In vitro assays.** Cytotoxicity was measured by a standard $^{51}$Cr release assay as previously described (10) against EL4 targets alone or EL4 cells that were incubated for 1 h with 0.1 nmol/L OVA257-264 at 37°C. Antigen-driven proliferation was determined as previously described with modifications (10). In brief, either naive OT-I spleen cells ($1 \times 10^5$ per well) or cytokine-expanded OT-I T cells ($2 \times 10^5$ per well) plus T-depleted mitomycin C–treated normal C57BL/6 spleen cells ($8 \times 10^4$ per well) were cultured with OVA257-264 in complete medium. [$^3$H]thymidine was added during the last 4 h of a 72 h culture. Similarly, we measured the expression of tumor antigen in various tissues by culturing for 3 d $5 \times 10^5$ naive OT-I cells with $10 \times 10^5$ mitomycin C–treated spleen or tumor cells isolated from tumor-bearing mice. [$^3$H]thymidine was added during the last 6 h of the cultures.

**Flow cytometry.** The following antibodies were used for flow cytometry for cell surface staining and were purchased from BD PharMingen: FITC-CD8 (53.6.7); FITC-CD45.2 (104); CyChrome-CD8 (53.6.7); and biotin-conjugated antibodies to CD44 (Pgp-1), CD62L (MEL-14), CD69 (H1.2F3), Ly-6C (AL-21), CD25 (IL-2R$\alpha$; 7D4), and CD122 (IL-2R$\beta$; T$\beta$1). We purchased CD127 (IL-7R$\alpha$; B12-1) from eBioscience. The cells were analyzed using LSR1 flow cytometer and CellQuest software (BD Biosciences) as previously described (10). Typically $1 \times 10^5$ and $1 \times 10^4$ events were collected for analysis of tissues of adoptively transferred mice or cultured cells, respectively. Dead cells were visualized with 7-amino-actinomycin D (7-AAD; BD PharMingen). For intracellular cytokine staining, the indicated cells ($1 \times 10^5$/mL) were cultured with OVA257-264 for 6, 24, or $48 \text{ h}$ and 1 mL of GolgiPlug (Brefeldin A) from BD Pharmingen was added during the last 4 h of the cultures. The cells were harvested and stained for surface marker expression, permeabilized using cytofix/cytoperm (BD PharMingen), and then stained with phycoerythrin–anti-IFN-$\gamma$ before fluorescence-activated cell sorting (FACS) analysis. To measure BrdUrd incorporation, a BrdUrd staining kit from (BD PharMingen) was used according to the manufacturer’s instructions.

### Results

**In vitro modeling of antigen-independent CD8$^+$ T memory development.** Past studies showed that the culture of naive OT-I T cells with OVA257-264 and IL-2 for 3 days generated activated T cells with properties expected of effector cells. Upon removing antigen and further culture of these CTLs with IL-7 or IL-15 for at least 48 hours, most cells exhibited properties consistent with central memory-like cells whereas extended culture in only IL-2 further promoted effector cell development (10). When OT-I CTLs
were cultured without cytokines, some conversion to memory was also noted (10). Although this finding suggests removal of antigen-promoted memory-like development, the poor cell recovery and viability under these culture conditions limit the interpretation of this finding.

One outcome of IL-15–induced signaling is up-regulation of Bcl-2 (24, 25), and high Bcl-2 expression is a normal property of memory CD8+ T cells (26). Therefore, we tested the contribution of IL-15 signaling, antigen removal, and cell survival to promote the development of central memory–like cells by initiating the cultures with Bcl-2 transgenic OT-I T cells (OT-I Bcl-2) as a means to provide an intrinsic survival signal. At the end of the 3-day priming cultures, the viability of the OT-I Bcl-2 T cells was high (>90%, data not shown), and importantly, remained high when these cells were further cultured for 2 days in the absence of antigen and exogenous cytokines as well as when cultured with IL-2 or IL-15 (Fig. 1A). As expected, without cytokine–dependent growth and survival signals, OT-I CTLs that were further cultured solely in the medium resulted in substantial cell death. Transgenic expression of Bcl-2 effectively prevented cell death, but unlike OT-I CTLs cultured with IL-2 or IL-15, cell expansion did not occur (data not shown).

Three days after naive OT-I or OT-I Bcl-2 T cells were cultured with OVA257-264 and IL-2, both types of OT-I T cells similarly expressed an activated phenotype with high expression of CD44, CD69, CD25, reduced expression of CD62L (Fig. 1B), and high CTL activity (Fig. 1C, day 3). The activated phenotype and cytolytic activity was maintained upon culture of these OT-I and OT-I Bcl-2 CTLs for 2 additional days with IL-2 (Fig. 1C, day 5). In contrast, when the day 3 OT-I or OT-I Bcl-2 CTLs were cultured for 2 additional days (day 5) with IL-15, they both resembled memory cells as CD62L, Ly-6C, and CD127 were up-regulated whereas CD25 and CD69 and CTL activity were down-regulated (Fig. 1B and C). Furthermore, simply culturing OT-I Bcl-2 CTLs for 2 additional days in medium without antigen or cytokines resulted in phenotypic and CTL activity that was largely comparable with the IL-15–induced memory-like cells (Fig. 1B and C). Another property of memory T cells is the induction of proliferative responses to lower levels of antigen than naïve T cells (27, 28). Therefore, we compared the proliferative responses by naïve OT-I T cells to the day 5 cultured cells as a function of OVA257-264 concentration (Fig. 1D). The OT-I and OT-I Bcl-2 T cells cultured with IL-2 did not appreciably proliferate to OVA257-264. This is likely due to the induction of apoptosis as we previously showed that IL-2 cultured OT-I CTLs underwent activation-induced cell death upon antigen re-encounter (10). Each of the memory-like populations, OT-I and OT-I Bcl-2 cultured in IL-15 or OT-I Bcl-2 cultured in medium, exhibited maximal proliferative responses to a lower concentration of OVA257-264 than naïve OT-I T cells, although the magnitude of the response by the naïve OT-I T cells was larger. This is likely due to the attenuation of antigen presentation in the IL-15 and medium-only cultures, but not the naïve cultures, because the memory cells efficiently lyse antigen-presenting cells (APC; ref. 29). Collectively, these data using OT-I Bcl-2 T cells indicate that CTLs rapidly acquire many key phenotypic and functional properties of memory-like cells derived by culture in IL-15 simply by removing antigen and providing an intrinsic survival signal. Additionally, in the presence of IL-15, the OT-I T cells expanded and more readily developed into a CD62Lhigh phenotype.

The effect of antigen on development of the memory phenotype in vitro. Our past work showed that residual antigen is not carried over from the 3-day priming culture when OT-I CTLs are further cultured with cytokines (10). To investigate the extent that antigen might affect the capacity of OT-I CTLs to develop into memory-like cells in vitro, day 3 OT-I CTLs were cultured with IL-15 in the absence or presence of OVA257-264 and APC. When cultured with only IL-15, or IL-15 and a low dose (0.01 nmol/L) of OVA257-264 that generated recall but not substantial primary responses (see Fig. 1D), the large majority of cells were CD62Lhigh, CD69+Ly-6C, and CD25low (Fig. 2), which resemble memory-like cells (see Fig. 1B). However, increasing the dose of OVA257-264 to 0.1 nmol/L, which stimulates substantial primary responses (see Fig. 1D), resulted in a cell phenotype expected for CTLs, that is, an increased fraction of CD62Llow cells and strong induction of CD69 and CD25. These data indicate that memory-like cell development does not require the complete absence of antigen in vitro but antigen levels must be sufficiently low.

Antigen-independent engraftment and programmed expansion during the effector to memory transition in vivo. After adoptive transfer of OT-I CTLs into normal recipient mice, a population of OT-I T cells persisted >60 days with phenotypic and functional properties of central memory cells (10, 22). As conversion to memory-like cells occurred quickly in vitro, we sought to determine the kinetics by which CTLs acquired memory cell traits in vivo. For these experiments, OT-I T cells were cultured with OVA257-264 and IL-2 for 3 days and the resulting CTLs were transferred to normal CD45.1 congenic wild-type (WT) C57BL/6 hosts, enumerating donor cells based on coexpression of CD8 and CD45.2. One day posttransfer, the frequency of OT-I T cells in the spleen and lymph nodes were ~0.5% of the total pool of CD8 T cells, which corresponded to 50,000 and 8,000 OT-I T cells, respectively (Fig. 3A). Approximately 7% of the CD8 T cells in the liver were OT-I cells, but the overall number of cells remained low, suggesting that nonlymphoid tissue was not a preferential site of OT-I trafficking. In this regard, a similar level of OT-I engraftment...
was noted for the lung (data not shown). The bone marrow also contained a very small fraction of donor OT-I T cells. From these data, we estimate that 1% to 2% of the initial donor inoculum engrafted the recipient mice. By day 3 posttransfer, the donor OT-I T cells markedly increased in the spleen, lymph nodes, and liver (Fig. 3A and B). Engraftment was similar over this time when OT-1 Bcl-2 CTLs were transferred, indicating that these levels of donor cells are not due to poor survival of the OT-I CTLs (Fig. 3B).

Because a relatively large number of CTLs were transferred, it was possible that engraftment was limited due to lack of space in appropriate in vivo niches as we used lymphoid cell replete recipient mice. However, when lower numbers of OT-I T cells were transferred, a proportionally lower number of OT-I T cells were detected in the spleen (Fig. 3C), lymph nodes, and liver (data not shown), suggesting that engraftment was limited based on the donor CTL rather than constraints on space.

The number of donor cells in lymphoid tissue and the liver increased for ~7 days postadoptive transfer after which the number of cells stabilized and then gradually declined over the next 60 days (Fig. 3D). To examine whether this increase was due to cell expansion rather than redistribution from some other anatomic site, 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE)–labeled OT-I CTLs were transferred to WT recipient mice and these cells were examined 1 to 3 days posttransfer. In the spleen, lymph nodes, and liver, the donor cells rapidly lost CFSE staining such that all cells were CFSE<sup>neg</sup> at 3 days posttransfer. This substantial proliferation closely corresponded to the expansion of OT-I T cells within these tissues. Thus, a population of OT-I CTLs that have recently encountered antigen in vitro continue to undergo proliferation without antigen in lymphoid and nonlymphoid tissues upon transfer in vivo, in a manner consistent with programmed expansion as described by others (10, 30–32).

**Kinetics of the effector to memory transition in vivo.** Our past work showed that the persistent OT-I T cells after adoptive transfer exhibited phenotypic and functional properties of central memory cells (10). However, the rapidity of the acquisition of such traits is not known. To investigate this point following the acute removal of antigen, we examined the cell surface phenotype and IFNγ production by adoptively transferred OT-I CTLs (Fig. 4B–D). For the few OT-I cells detected 24 hours after transfer in the spleen (Fig. 4B) and lymph nodes (data not shown), CD25 and CD69 were already down-regulated whereas IL-2R<sup>α</sup> was up-regulated. However, a more pronounced memory phenotype was detected 3 days after transfer, as the large majority of cells were CD44<sup>high</sup>, CD62L<sup>high</sup>, CD69<sup>neg</sup>, CD25<sup>neg</sup>, IL-7R<sup>h</sup>, and IL-2R<sup>α</sup>high; by 14 days, essentially full conversion to central memory phenotype was noted (Fig. 4B). In contrast, many of the persistent cells in the liver remained CD62L<sup>neg/low</sup>, a property expected for effector-memory cells (Fig. 4C).

With respect to IFNγ production (Fig. 4D), when assayed 6 hours after OVA<sub>257–264</sub> (0.1 nmol/L) stimulation <em>ex vivo</em>, a relatively high fraction of donor OT-I T cells produced IFNγ at 3 and 7 days posttransfer. After this time, the frequency of IFNγ<sup>+</sup> cells substantially decreased, with a stable population of 20% to 30% of the cells being IFNγ<sup>+</sup> at 21 to 60 days posttransfer. In contrast,
when IFNγ production was assessed 24 hours after ex vivo stimulation, 70% to 80% of the donor OT-I T cells were IFNγ+, which was significantly higher than detected after activation of naïve T cells, which required 48 hours for maximal IFNγ production (Fig. 4D). Thus, 3 days after adoptive transfer, most of the donor OT-I T cells exhibited rapid recall responses to antigen and expressed a cell surface phenotype that largely resembled central memory cells.

The effect of persistent tumor antigen on the effector to memory cell conversion. The above experiments show that CTLs rapidly develop into central memory cells when acutely placed into environments that lacked antigen. To directly examine the effect of antigen on the phenotypic conversion to memory T cells in vivo, OT-I CTLs were adoptively transferred into mice bearing the OVA-expressing EL4 tumor E.G7. For these experiments, E.G7 was allowed to grow in CD45.1 congenic C57BL/6 mice to a measurable mass of 50 mm³, at which time mice received $0.1 \times 10^7$ or $1 \times 10^7$ OT-I CTLs whereas E.G7 was not eliminated in the mice that received $0.1 \times 10^7$ CTLs. Here, it remained detectable but at a somewhat lower mass than in untreated tumor-bearing mice (Fig. 5A). At 10 days posttransfer, OT-I T cells were detected in the spleen and lymph nodes that were largely proportional to the number of cells transferred (Fig. 5B). For the mice that received $0.3 \times 10^7$ or $1 \times 10^7$ OT-I CTLs and cleared the tumor, the phenotype of the persistent cells resembled central memory cells, that is, CD44high, CD62Lhigh, CD69neg, CD25neg, Ly-6Chigh, CD127high, and CD122high (Fig. 5C). Furthermore, when these mice were rechallenged with E.G7, the persistent OT-I cells prevented the establishment and growth of tumor (data not shown). In contrast, the OT-I T cells detected in the lymph nodes of the recipient mice that did not clear E.G7 displayed lower expression of CD62L, CD122, and CD127 and more cells (~30%) expressed CD69. This pattern of surface marker expression indicated that these cells retained the phenotype of an activated effector cell. Whether the OT-I T cells with an activated phenotype
in these lymph nodes reflected local activation by tumor antigen or recent entry of activated OT-I T cells from the tumor site remains to be determined. Thus, in the presence of a continuous tumor burden over a 10-day period, the conversion of CTLs to central memory phenotype was somewhat impaired and inefficient tumor rejection correlated with inefficient conversion to a memory-like cell surface phenotype.

As OT-I CTLs acquired many properties of memory cells 3 days after transfer into antigen-free mice, we assessed the phenotype and functional properties of OT-I CTLs on day 3 posttransfer while mounting an antitumor response to E.G7. When compared with mice without tumor analyzed in parallel, the OT-I T cells within the lymph nodes and spleen in the tumor-bearing recipients were present at a similar fraction (Fig. 6A) and expressed a similar phenotype that resembled memory cells (i.e., CD44\textsuperscript{high}, CD62L\textsuperscript{high}, Ly-6C\textsuperscript{high}, CD122\textsuperscript{high}, CD69\textsuperscript{neg}, and CD25\textsuperscript{neg}; Fig. 6B). One exception was CD127, which was lower on the OT-I T cells in tumor-bearing mice. In contrast, the OT-I T cells found within the s.c. tumor site resembled effector cells in that most were CD62L\textsuperscript{low} and many were CD69\textsuperscript{+}. Furthermore, the OT-I T cells in the spleen and lymph nodes of the E.G7-bearing mice developed IFN\textgamma recall responses to OVA\textsubscript{257-264} that were greater than that seen in the OT-I T cells from non–tumor-bearing mice (Fig. 6C). Although E.G7 was present 3 days after the transfer of OT-I CTLs when these mice were evaluated, we confirmed that the OT-I CTL rejected E.G7 in a parallel group of mice (data not shown). Thus, these data indicate that some CTLs rapidly convert to memory cells, concurrent with an effector response, before complete clearance of tumor antigen.

Unlike most infectious models, such as Listeria and LCMV, where antigen is disseminated systemically (33), the distribution of tumor antigen was expected to be localized to the tumor site (34). To determine the distribution of OVA in tumor-bearing mice, we tested cells isolated from the spleen or the tumor site to serve as APC and induce proliferation by naive OT-I T cells (Fig. 6D). Spleen cells from the tumor-bearing mice induced very low proliferation by naive OT-I T cells, similar to OT-I spleen cells that were stimulated with 0.01 nmol/L OVA\textsubscript{257-264}. In contrast, the E.G7 cells from the tumor site induced substantial proliferation that was similar to the proliferative responses seen after OT-I spleen cells were cultured with 0.1 to 1 nmol/L OVA\textsubscript{257-264}. These findings indicate that very little tumor antigen is present within secondary lymphoid tissue, consistent with observations by Boissonnas and colleagues (34) using a similar E.G7 tumor model. Thus, the low level of antigen in the spleen and lymph nodes was permissive for the generation of memory cells whereas the high level of OVA at the tumor site favored the continued activation of the effector OT-I T cells.

![Figure 5](https://example.com/figure5.png)

**Figure 5.** Antitumor activity and memory T-cell development after transfer of OT-I CTL into tumor antigen–bearing mice. E.G7 was injected s.c. in the abdomen of normal CD45.1 congenic C57BL/6 mice. After the tumor grew to \(50 \text{ mm}^3\), the mice were left untreated or adoptively transferred with the indicated number of OT-I CTLs. Shown are (A) tumor growth after OT-I CTL transfer or (B) the frequency and (C) the cell surface phenotype of the persistent donor OT-I cells 10 d posttransfer. Points and columns, representative or mean of 3 to 5 mice per group; bars, SD.
Discussion

Adoptive T-cell immunotherapy remains an attractive approach to eliminate tumors or eradicate certain viral infections. A potential advantage of adoptive T-cell therapy is the ability to control the induction of the T-cell response without the inherent complexity of inducing an immune response in vivo. Patients with a significant tumor burden or persistent virus are often immunosuppressed, decreasing the likelihood of inducing the desired immune response. This study and our past work (10, 22) support the view that culture conditions that yield long-term persistent T cells upon adoptive transfer are highly effective in antitumor therapy. Furthermore, the acquisition of memory cell traits by the activated CD8+ T cells is predictive for their persistence and successful immunotherapy. Although short-term effector cells have been shown to be more effective in eradicating melanoma than fully differentiated CTLs (13), the tumor-bearing mice required lymphoid depletion conditioning to promote engraftment and in vivo application of peptide vaccine and IL-2. Here, we found that CTL eradicated an established tumor mass, without a need for prior lymphoid depletion, vaccination, or application of exogenous cytokines, simplifying adoptive T-cell therapy for at least some tumors.

Previously, we showed that short-term effector CTLs or long-term cultures of activated CD8+ T cells in IL-15 were superior to cells cultured in IL-2 for an extended period in their persistence upon adoptive transfer to lymphoid-replete normal mice and ability to reject a tumor challenge (10, 22). Moreover, the IL-15 cultured cells exhibited many properties of central memory cells (10). Here, we establish that the removal of antigen was largely responsible for the acquisition of memory-like traits in vitro rather than directed by IL-15 signaling. Furthermore, the persistent T cells after adoptive transfer of short-term OT-1 CTL exhibited phenotypic and functional properties of central memory cells (10). We show here that these short-term effector cells readily rejected an established tumor while rapidly acquiring properties of memory cells.

As short-term CTLs were previously shown to yield the highest proportion of persistent memory-like CD8+ T cells upon adoptive transfer, we were especially interested to determine the basis for this population of T cells. Upon adoptive transfer into WT mice, these OT-1 CTL developed into both effector memory-like and central memory-like cells and this was obvious as early as 3 days after adoptive transfer. The OT-1 T cells that were detected in the lymphoid organs displayed cell surface characteristics of central memory T cells, whereas the cells detected in the nonlymphoid organs displayed a cell surface phenotype, characteristic of effector memory T cells, recapitulating the development of memory cell subsets following an acute infection. These transferred cells persisted long term in the absence of antigen and were detected at least 60 days posttransfer.

We found a striking dichotomy with respect to the efficiency by which CTLs convert to memory-like cells in vitro versus in vivo. Essentially all OT-1 CTLs convert to memory-like cells upon transgenic expression of Bcl-2 or culture with IL-15, which is known to enhance Bcl-2 levels in memory cells (35). These in vitro data are consistent with the view that all CTLs have the potential to readily acquire properties of memory cells. However, upon adoptive transfer of these same CTLs to normal antigen-free mice, only a minor fraction engrafted. These cells then rapidly expanded, probably through programmed expansion, and acquired many
phenotypic and functional properties of memory cells, yielding a significant population of donor cells by 7 days posttransfer. These results are consistent with the notion that there is a subset of CTL that preferentially develop into memory cells in vivo. At this juncture, we do not have any information concerning what might identify such a CD8+ T-cell subset, as it seems that all CTLs have intrinsic potential to become CD8+ memory cells in vitro. We speculate that the distinguishing property might reflect expression of a favorable chemokine or cytokine receptor promoting localization within a defined niche and/or cell survival. The observation that IL-7Rαhigh cells at the peak of a LCMV infection are enriched in cells destined to become memory cells is consistent with this view (36).

Our culture system using IL-15 or the adoptive transfer of short-term CTL into normal mice closely approximates CD8+ memory T-cell development as defined by in vitro infectious systems (18, 31). Another aspect of this study is that we have model systems that are useful to study early events as effector CTLs develop into memory cells. Many studies have correlated memory CD8+ T-cell production with antigen clearance after infections (36–38), but it is complex to directly explore the importance of antigen removal for memory development or assess the rapidity by which memory cell traits are acquired. Our model systems provide information directly relevant to these two issues. When CTLs were placed in an antigen-free environment in vitro or in vivo, many phenotypic and functional properties of CD8+ memory T cells were acquired 48 to 72 hours later. Our finding that CTLs rapidly acquire memory cell properties might be viewed as contradictory to the notion that CD8+ memory T-cell development occurs slowly over weeks after the antigen is eliminated (31, 36, 38). However, just as with LCMV infection (39), high levels of IL-7Rα and CD62L expression required more time than down-regulation of CD69 and CD25 or up-regulation of CD44 after OT-I CTLs were transferred to antigen-free mice. Thus, our data suggest that memory T-cell development is bimorphic with initial rapid acquisition of key memory cell traits whereas others require more time. Another important point of our study is that CD8+ memory T-cell conversion also occurred in vitro and in vivo in the presence of antigen; in the latter instance, the conversion is associated with a growing tumor. It is extremely difficult to quantify antigen dose within the niches that memory cells develop in vivo. By modeling CD8+ T memory in vitro, however, a marked cutoff was found in which memory development was permissive, that is, a dose of OVA257-264 that efficiently promoted a recall, but not a primary response. At the higher dose of OVA257-264, the OT-I T cells retained an effector cell phenotype. Furthermore, the antigen load within the spleen of mice that contained a palpable s.c. tumor of 50 mm3 was relatively low based on the magnitude of proliferative responses by naïve OT-I T cells induced by APC from these spleens. Therefore, it is likely that the antigen dose within the lymphoid compartment in vivo dictates whether CTLs convert to memory T cells. In the case of EG7 where the CTL rejected this tumor, the amount of OVA257-264 was apparently too low to prevent rapid memory conversion in the lymph nodes and spleen. However, when a relatively small number of OT-I CTL was transferred, which did not reject EG7, the tumor burden and by extension OVA levels increased and memory conversion was impaired.

Our findings support the recent view that it is the quality, not simply the quantity, of T cells for adoptive T-cell therapy that predicts successful therapy (40). In several other studies, short-term cultures of T cells were also found to be very effective in eradicating large preexisting tumor burdens (11, 13). Alternatively, culture of antigen-activated CD8+ T cells in IL-15 rather than IL-2 was also more favorable for persistence and antitumor activity in vivo (10, 12, 15). Effector CD8+ T cells that express very potent effector function after extended culture in IL-2 were shown to be less efficacious in adoptive therapy in part due to low expression of CD62L, less proliferative potential, and greater sensitivity to apoptosis (13). These findings and our results indicate that the best population of T cells for adoptive T-cell therapy is one that has been optimally, but briefly, activated in vitro for 3 days. Consistent with this view, preliminary work suggests that IL-15–expanded memory-like T cells may be somewhat less effective for adoptive therapy of established E.G7 tumors (not shown). Generating a sufficient number of short-term antigen-specific cells is easy using TCR transgenic T cells. This will be more challenging for true tumor antigens as the antigen-specific precursor frequency is likely to be low. Thus, a short culture period remains compatible with adoptive T-cell therapy but likely will need to be combined with approaches to redirect the specificity of the CTL, for example, by forced expression of a TCR that recognizes a tumor antigen or by the use of bispecific antibodies (41–45). Furthermore, if continued expansion is required for adoptive transfer, use of IL-15 as a growth factor seems preferable over IL-2.

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Modeling the CD8⁺ T Effector to Memory Transition in Adoptive T-Cell Antitumor Immunotherapy

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