Harnessing cytotoxic CD8+ T cells against neoplastic lesions has been a major goal of anticancer immunotherapy. The clonal expansion and activation of these cells are triggered by antigen-specific interactions between their T-cell receptors (TCRs) and the cognate tumor-associated antigen (TAA) displayed in complex with MHC molecules on the surface of malignant or antigen-presenting cells. Surprisingly, a recent report has shown that cytokine-based systemic immunotherapy can trigger the antitumor functions of memory CD8+ T cells in the absence of specific antigenic stimulation. Following the co-administration of interleukin (IL)-2 and a CD40-targeting agonist antibody to mice, memory CD8+ T cells underwent a rapid expansion, upregulated killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D) and granzyme B, and acquired broad lytic functions. These cells failed to upregulate programmed cell death 1 (PD1), best known PD-1) and CD25, suggesting that their activation was independent of TCR signaling. Furthermore, the authors demonstrated that antigen specificity is not mandatory for the expansion and antitumor activity of memory CD8+ T cells as elicited by systemic immunotherapy in TCR-transgenic mice. Immunotherapy-activated ovalbumin (OVA)-specific memory CD8+ T cells were indeed capable of lysing OVA+ as well as OVA− tumors in vitro and also mediated significant antineoplastic effects in vivo. Taken together, these findings indicate that memory CD8+ T cells activated by IL-2 and CD40 signaling can acquire an unusual innate-like phenotype and become capable of mounting antigen-independent cytotoxic responses against tumor cells. Human T cells with a similar phenotype were observed in melanoma patients upon localized imiquimod-based immunotherapy, suggesting that such immune responses may be conserved across species. Recent studies have demonstrated that bacterial, viral and parasitic infections can also trigger memory CD8+ T cells to proliferate and become potent effector cells in the absence of specific antigenic stimulation via a process of natural inflammation known as “bystander” activation. In a Listeria monocytogenes (Lm) immunization mouse model, Soudja et al. showed that Lm-specific memory CD8+ T cells can acquire strong effector functions and expression of activation markers without the requirement for antigen recognition. Such activation and differentiation of memory CD8+ T cells into potent effector cells, which contribute to anti-bacterial immunity, was shown to be orchestrated by IL-15 and IL-18, which are secreted by inflammatory monocytes upon exposure to various classes of microbial pathogens. Along similar lines, Chu et al. subsequently showed that bystander-activated memory CD8+ T cells can control the early pathogen load by killing target cells through an NKG2D-dependent mechanism, importantly mediating anti-influenza responses prior to the initiation of

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adaptive immunity. In a mouse influenza model, Tietze et al. found that adoptively transferred OVA-specific memory CD8+ T cells proliferated in the lungs and displayed increased levels of NKG2D, but not CD25, in response to influenza infection. In this setting, the intranasal blockade of NKG2D resulted in a significant increase in viral replication in the early phase of infection. These studies demonstrate that microbial pathogens induce a rapid, antigen-independent expansion of memory CD8+ T cells at the site of inflammation, resulting in the elicitation of NKG2D-dependent innate immune responses against infectious agents.

In studies described above, either multiple immunostimulatory proteins or inflammatory mediators were required to expand and activate memory CD8+ T cells in the absence of specific antigenic stimulation. Conversely, we have recently shown that the systemic administration of an IL-15 superagonist complex, ALT-803 (Fig. 1), is sufficient to trigger memory CD8+ T-cell responses that mediate robust antitumor effects in several mouse models of myeloma. ALT-803 contains a mutant form of interleukin-15 (IL-15N72D) associated with a dimeric IL-15 receptor α chain sushi domain (IL-15RαSu)-IgG1 Fc fusion. The N72D substitution confers to IL-15 increased affinity for the IL-2 receptor β chain (IL-2Rβ) and enhanced biological activity. In addition, association of IL-15N72D with IL-15RαSu further improves the biological activity of IL-15 in vivo, resulting in the potent activation of IL-2Rβ/γ-bearing natural killer (NK) cells and T lymphocytes. In myeloma-bearing mice, ALT-803 promoted the rapid expansion of memory CD8+ T cells but not naïve CD8+ T lymphocytes. Such memory CD8+ T cells secreted high levels of interferon γ (IFNγ) and unregulated killer cell lectin-like receptor subfamily K, member 1 (KLRK1, best known as NKG2D) but not of programmed cell death 1 (PD1) and CD25, on their surfaces. ALT-803-activated cells also mediated nonspecific cytotoxicity against myeloma cells and other tumor cells, via a mechanism that was partially dependent on IFNγ. By activating such a response, ALT-803 was capable of eliminating well-established myelomas from the bone marrow and significantly prolonging the survival of tumor-bearing mice. Short-term ALT-803 treatment also provided tumor-bearing mice with protective immunity against a subsequent inoculation of myeloma cells. This protective response appeared to rely on CD8+ T lymphocytes. Presumably, ALT-803 treatment stimulated naïve and/or memory CD8+ T cells specific for tumor-associated antigens (TAs) to acquire effector functions against a subsequent tumor challenge.

The treatment of mice bearing 5T33 or MOPC-315 myelomas with ALT-803...
but not IL-15, rapidly eliminated malignant cells from the bone marrow and prolonged survival, often curing mice, in a CD8+ T-cell dependent manner.\(^7\) NK cells were not required for such anti-myeloma activity. Conversely, the ALT-803-mediated elevation of CD8+ T cells in the bone marrow correlated with therapeutic responses, supporting the hypothesis that ALT-803 induces innate-like memory CD8+ T cells that efficiently kill myeloma cells. Furthermore, as it also activates NK cells in vitro and in vivo,\(^3\) ALT-803 might have the potential to elicit broad innate immune responses against neoplastic and infected cells.

Finally, we observed that the curative, short-term administration of ALT-803 to tumor-bearing mice provided them with a CD8+ T cell-dependent protection against a subsequent rechallenge with myeloma performed months later.\(^7\) These findings suggest that ALT-803 also elicits its efficient adaptive immune responses, resulting in the generation of long-term T cell-based antitumor immunity. Thus, ALT-803 stands out as a potent immunostimulant that is capable of simultaneously activating the innate and adaptive arms of the immune system to elicit both rapid and long-lasting protective responses against infectious or neoplastic challenges to the host.

**Disclosure of Potential Conflicts of Interest**

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