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Anticancer Tc9 cells

Long-lived tumor-killing T cells for adoptive therapy

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IL-9-producing cytotoxic T (Tc9) cells represent a unique CD8+ T-cell subset. These long-lived immune cells possess the capacity to acquire effector function and home to tumor tissues after adoptive transfer. IL-9 is indispensable for Tc9-mediated superior antitumor response. These findings are highly significant and crucial to achieve advances in T cell-based adoptive therapies.

Adoptive cell therapy (ACT) to treat cancer is transitioning from a promising possibility to a successful reality. Encouraging clinical data suggest that ACT will be a safe and effective treatment modality for advanced melanoma and other tumors. Unfortunately, however, a complete response occurs infrequently, with most patients experiencing recurrence.[1] Current clinical ACT protocols use ex vivo expanded tumor-specific type-1 CD8+ cytotoxic (Tc1) cells generated by the application of high doses of IL-2. Although IL-2 can augment antigen-induced CD8+ T-cell expression of the cell-fate regulatory transcription factor eomesodermin (Eomes) and promote acquisition of the CD8+ effector T-cell cytolytic phenotype, these T-box 21^{high} (Tbx21, better known as T-bet) expressing Tc1 cells display terminal effector features and have a short lifespan after ACT.[2] It is increasingly evident that advances in ACT depend upon transfer of CD8+ T cells possessing enhanced persistence, appropriate homing, and fully cytolytic effector function in vivo.

Interleukin 9 (IL-9) is a common receptor γ chain family cytokine that regulates a broad range of immune responses. In studies of murine cancer models, we have reported that IL-9-producing T helper type 9 (Th9) cells not only inhibit tumor progression but also promote greater tumor clearance than T helper type 1 (Th1) cells. We demonstrated that Th9 cells maintain an IL-9-producing, non-cytolytic phenotype that promotes a strong host CD8+ cytotoxic T lymphocyte (CTL)-mediated antitumor immune response.[3,4] We found that Th9 cells do not appear to be directly involved in the cytolysis of tumor cells, but rather, the IL-9 that they produce provokes a unique inflammatory environment in tumor tissues favoring CD8+ CTL activation by enhancing antigen-presentation in tumor draining lymph nodes. IL-9 is crucial in the subsequent homing of the activated tumor-specific CTLs to tumor sites whereupon they exert their killing functions. Considering that Th9 cells are not cytolytic effector cells, we were curious as to whether IL-9-producing CD8+ T (Tc9) cells could be generated, cells that could potentially be the optimal CD8+ T-cell subset for ACT. To examine this possibility, we primed naïve CD8+ T cells under Th9-polarizing conditions.[5] This unique priming induced fewer CTL cell-expressing granzyme B (GrzB), Eomes, T-bet, and interferon-γ (IFNγ), and instead promoted the development of IL-9-producing Tc9 cells with upregulated expression of *Irf4* and *Pu.1*, transcription factors that typically determine Th9-cell lineage differentiation. As expected, these artificially in vitro differentiated Tc9 cells displayed significantly weaker cytolytic activity against target tumor cells as compared with Tc1 cells due to their lack of typical CTL-expressing molecules. Despite their diminished cytolytic activities in vitro, Tc9 cells are a new subset of candidate CD8+ T cells that may be more efficacious for anticancer ACT regimens.

Current advances in ACT have renewed our understanding of the use of terminally differentiated end-effector CD8+ cells, those harboring the greatest potential to release IFNγ and effectively lyse targets in vitro but exhibiting limited function after transfer in vivo.[6,7] The 3 major determinants of successful ACT are cell persistence, appropriate homing, and acquisition of target killing function in a pathophysiological context. In testing whether Tc9 cells meet these requirements, we unexpectedly found that, although less cytolytic ex vivo relative to Tc1 cells, adoptive transfer of tumor-specific Tc9 cells elicited a significantly greater antitumor response against large established melanoma (B16 and B16-OVA) and colon (MC38-gp100) tumors in vivo. These exciting antitumor effects were associated with substantially enhanced persistence of the transferred Tc9 cells throughout the experiment, a homeostatic shift resulting more from greater resistance to apoptosis rather than enhanced proliferative capacity. In the case of Tc1-based ACT, tumor-bearing mice appear to always "run out" of

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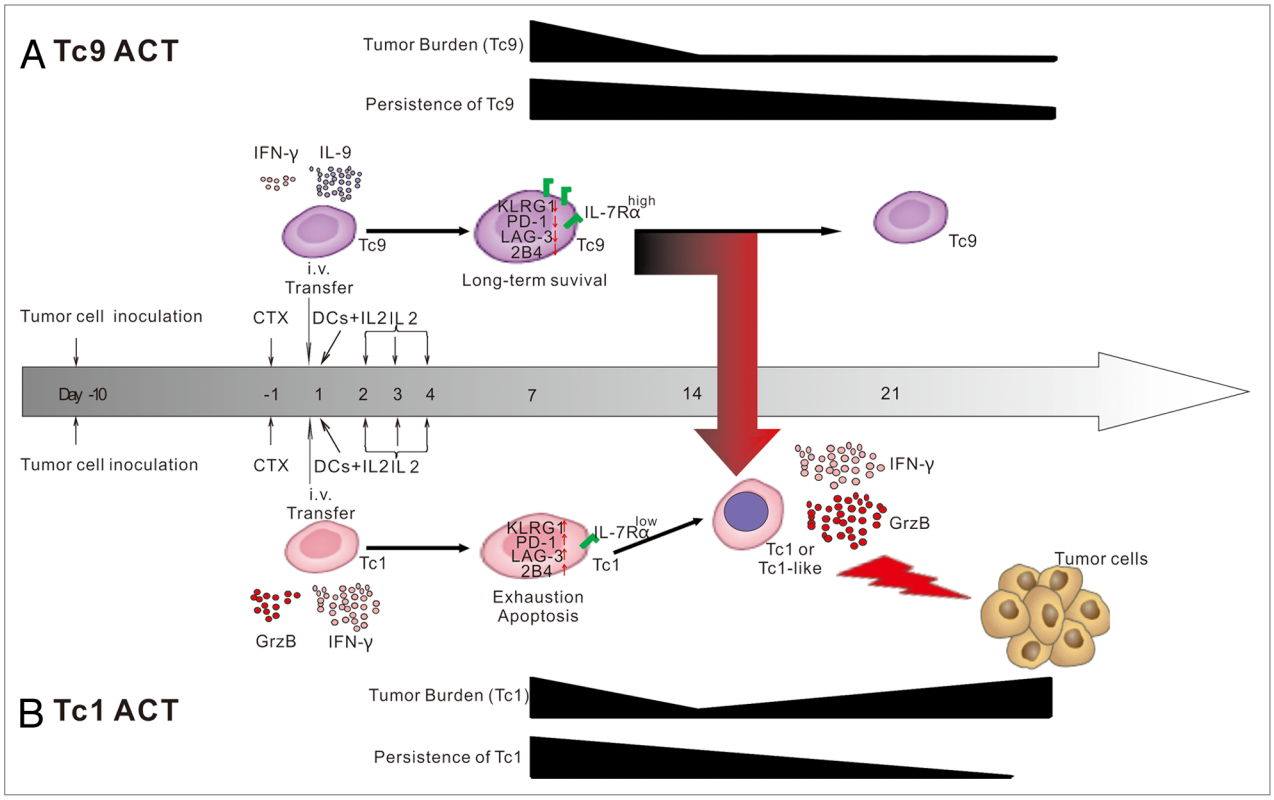

Figure 1. Antitumor adoptive cell therapy (ACT) with IL-9-expressing CD8+ cytotoxic T cells vs. type-1 CD8+ T cells. (**A and B**) Mice bearing 10-d advanced tumors were transferred with (**A**) Il-9 polarized tumor-specific CD8+ cytotoxic T cells (Tc9) or (**B**) type-1 CD8+ T cells (Tc1) cells, in combination with the indicated adjuvant therapies. Approximately 14 d after transfer, non-cytolytic IL-9-producing Tc9 cells displayed a less exhausted phenotype and converted into large numbers of cytolytic Tc1-like cells. The induction of large numbers of Tc9-derived interferon-γ (IFNγ) and granzyme B (GrzB) expressing-effector cells contributed to the sustained therapeutic effects of Tc9 adoptive cell therapy (ACT). In contrast, Tc1 cells acquired a signature of terminal differentiation with high expression of exhaustion-phenotypic markers, leading to the failure of homeostatic proliferation, dysfunction, and depletion of these cells in vivo. Consequently, Tc1 ACT can only meditate a temporary tumor regression.

transferred CD8+ T cells between 2 and 3 wk after transfer, corresponding to the time when tumors recur with aggressive growth after initial shrinkage. In addition to long-term persistence in vivo, gain-of-function is another key feature of effector T cells that successfully mount an antitumor response. Indeed, Tc9 cells display a less exhausted "younger" cell phenotype with very low surface expression of exhaustion/inhibitory molecules, such as killer cell lectin-like receptor subfamily G, member 1 (KLRG-1), programmed cell death 1 (PD-1), lymphocyte activation gene 3 (LAG3), and CD244, natural killer cell receptor 2B4. Importantly, Tc9 cells are long-lived cells capable of maturational plasticity and can switch into IFNγ- and GrzB-secreting effector cells about 2 wk after transfer. Notably, adjuvant therapies, including chemotherapy-induced lymphopenia, dendritic cell (DC) vaccination, and exogenous IL-2 administration, are crucial for this Tc9-to-Tc1-like cytolytic effector cell conversion, possibly because they provide a highly activating environment to promote Tc1-like lineage specification. Withdrawing any of these adjuvant therapies can correspondingly decrease efficient conversion of Tc9 cells to Tc1-like lineage, in turn making Tc9-ACT less effective in controlling the growth of large, advanced tumors. **Figure 1** depicts the distinct phenotype and therapeutic outcome of Tc9- vs. Tc1-based ACT.

The production of IFNγ, a quintessential feature of cytolytic T cells, is critical to the antitumor efficacy of Tc1, Tc17, Th1, and Th17 cells.[8-10] Tc9 cells convert, or flip the cytokines they produce after transfer. Previously, Tc9 cell-produced IFNγ rather than IL-9 was thought to be the more crucial cytokine dictating Tc9 cell-mediated antitumor responses. However, our results clearly demonstrate that IL-9 should never be considered as only a byproduct of Tc9 cells in regard to their antitumor efficacy. Unexpectedly, we found that anti-IL-9 antibody treatment largely abrogated tumor rejection, whereas IFNγ neutralizing antibodies only slightly affected the antitumor activities of Tc9 cells, as compared with isotype control. Further analysis revealed that, in the absence of IL-9, Tc9 cells largely failed to migrate into tumor tissues to exert long-lasting therapeutic effect, as shown by a significantly decreased number of tumor-infiltrating but not splenic GrzB-positive Tc9-derived cytolytic cells. In sum, IL-9 production allows significant numbers of Tc9-derived antitumor lymphocytes to home appropriately to tumor beds, enabling them to efficiently seek out and destroy target cancer cells in vivo. Future work is needed to validate these findings in human Tc9 adoptive cell therapy settings.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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