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A clinically useful approach to enhance immunological memory and antitumor immunity

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Persistence of vaccine-induced immune responses, not the initial magnitude, best correlates with protective antitumor immunity. In mice, oligonucleotide aptamer-targeted siRNA inhibition of mammalian target of rapamycin (mTOR) activity in activated CD8+ T cells promotes their differentiation into functionally competent memory cells leading to enhanced antitumor immunity, a protective effect superior to that of non-targeted administration of the mTOR inhibitor rapamycin.

The quest for developing an immune-related treatment modality to control cancer progression has received formal validation with the US. Food and Drug Administration approval of Sipuleucel-T, a cell-based anticancer vaccine, and ipilimumab, an immune stimulatory antibody, for the treatment of prostate cancer and melanoma, respectively. Given their modest therapeutic impact, the goal and challenge of cancer immunotherapeutic research is how to elicit increasingly potent antitumor responses that will control and even reverse tumor growth in the cancer patient. The development of cancer vaccines has been largely predicated on the assumption that the initial magnitude of the vaccine-induced immune response, namely the number of immune effector cells elicited shortly after vaccination, will dictate the constraint of tumor growth. Nevertheless, a growing body of evidence suggests that the therapeutic potential of a (vaccine-induced) antitumor immune response is more a function of its ability to persist, and less a function of its initial magnitude.[1] The implications are that a weak yet persistent antitumor immune response could be clinically more effective than a potent transient response.

Following vaccination, the majority of activated T cells will differentiate into short-term effector cells, whereas only a small proportion will develop into long-lasting memory cells. Therefore, the challenge facing researchers is how to redirect the tumor vaccine activated T cells toward the memory pathway, and further, how to do that using a clinically useful, cost effective, and broadly applicable approach. The differentiation of activated T cells into effector or memory cells is regulated by a host of extrinsic factors as well as intracellular mediators (**Fig. 1**). Interestingly, inhibition of many of the intracellular mediators of effector differentiation such as mTOR, Blimp-1, or glycolysis metabolites, not only prevented their development into short-lived effector but also redirected the activated T cell to differentiate into long-lasting memory cells.[2-4]

In a seminal study Araki et al. have shown that treatment of lymphocytic choriomeningitis (LCMV)-infected mice with the mTOR inhibitor rapamycin led to the generation of a potent memory CD8+ T cell response, opening the door for the use of pharmacological agents to enhance memory responses in human patients.[5] Nonetheless, the use of pharmacological agents in clinical settings is fraught with limitations and unintended consequences reflecting their often pleiotropic effects. For example rapamycin, an approved immunosuppressive drug, can skew the differentiation of CD4+ T cells into immunosuppressive regulatory T cells and/or the polarization of dendritic cells to become tolerogenic antigen presenting cells, the last thing one wants to do in the setting of vaccination.[6]

It stands to reason that limiting the inhibition of intracellular mediators of effector differentiation to CD8+ T cells will obviate the aforementioned limitations. With this in mind we have recently described a murine approach to inhibit mTORC1 (mammalian target of rapamycin complex 1) function using siRNA targeting a key component of mTORC1 referred to as raptor (RPTOR, regulatory associated protein of mTOR) specifically in activated CD8+ T cells via conjugation to an oligonucleotide aptamer that binds specifically to 4–1BB, a costimulatory receptor transiently expressed on activated CD8+ T cells.[7] siRNAs are ideally suited to inhibit the function of intracellular products that are not accessible to antibodies, often referred to as "nondrugable" targets, and the short chemically synthesized oligonucleotide aptamer ligands

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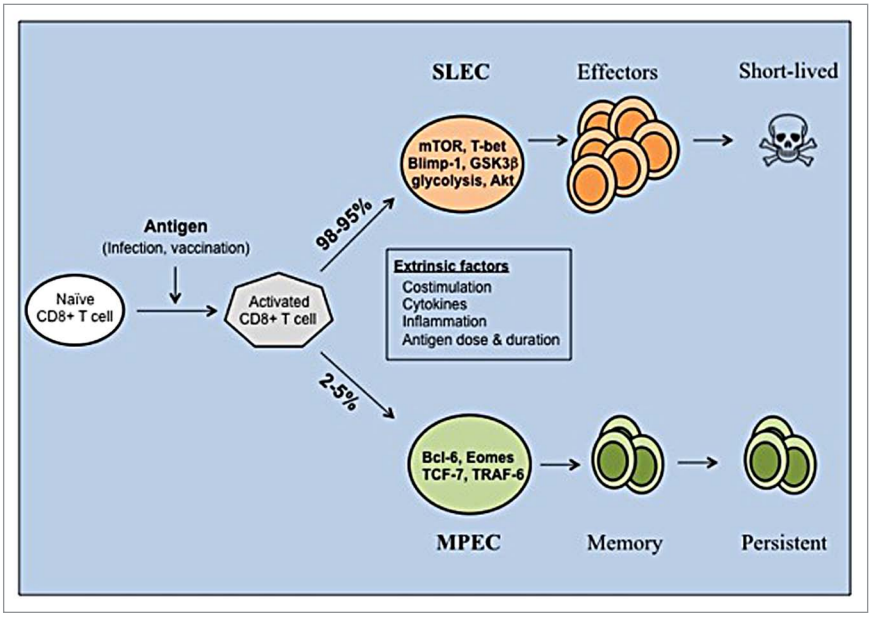

Figure 1. Differentiation of antigen activated CD8+ T cells into terminally differentiated effectors or long-lasting memory cells. The majority of naïve CD8+ T cells activated in the course of infection or in response to vaccination differentiate into short-lived effector cells (SLECs) that give rise to terminally-differentiated effector cells secreting proinflammatory cytokines and/or endowed with MHC class I-restricted cytotoxicity. A small proportion of activated T cells differentiate into memory precursor effector cells (MPECs) that give rise to long-lasting memory cells capable of responding to subsequent antigenic challenges. Differentiation of activated T cells into effector or memory cells is regulated by extrinsic factors as well as intracellular mediators expressed in the activated T cells. The relative concentration of intracellular mediators, presumably dictated by the nature of the extrinsic factors prevailing during priming, will determine the proportion of effector and memory cells generated. Abbreviations: mTOR, mechanistic target of rapamycin; T-bet (TBX21), T-box transcription factor 21; Blimp-1 (PRDM-1), PR domain zinc finger protein 1; GSK3β, glycogen synthase kinase 3 β; Akt, thymoma viral proto-oncogene 1; Bcl-6, B cell lymphoma protein 6, Eomes, eomesodermin; TCF-7,transcription factor 7; TRAF-6, TNF receptor associated factor 6.

offer potentially significant advantages over monoclonal antibodies ligands in terms of synthesis, cost, chemical conjugation, and possibly lack or reduced immunogenicity.[8] We have first shown that aptamer-targeted raptor siRNA delivery is remarkable effective and specific, at least 60% of the activated CD8+ T cells showing evidence of downregulated mTORC1, but not mTORC2 (mammalian target of rapamycin complex 2) a related serine-threonine signaling complex which does not require raptor. Contrasting with the relatively efficient delivery of siRNA to hepatocytes or solid tumor cells, so far the delivery of siRNAs to hematopoietic cells in vivo has been notoriously inefficient,[9] such that aptamer targeting could represent a quantum leap in using RNAi to manipulate the normal and malignant hematopoietic system.

Aptamer targeted downregulation of raptor and mTORC1 activity led to enhanced generation of memory CD8+ T cells capable of responding to subsequent antigenic challenge by proliferation and elaboration of cytotoxic effect functions. Notably, the rapamycin-generated memory CD8+ T cells exhibited, an apparently non cell-autonomous, defect in their cytotoxic effector functions. Consistent with an enhanced memory response, treatment of mice with the aptamer-siRNA conjugates engendered a superior protective immunity in both prophylactic and therapeutic models that was superior to that of rapamycin (as shown in the former). It is conceivable that the striking differences between rapamycin and aptamer-mediated siRNA treatment on immune memory and antitumor immunity are a reflection of the limitations of non-targeted vs. targeted drug delivery, the underlying premise of this approach. We don't know factually, and there is no reason to think conceptually, that mTOR inhibition is the best route to promote immune memory. The aptamer-targeted siRNA approach can be readily extended to other targets such as Blimp-1 (PR domain zinc finger protein 1, PRDM-1), CD25 (α chain of the IL-2 receptor, IL2RA), T-bet (T-box transcription factor 21, TBX21), or glycolysis. One target we are particularly interested in inhibiting is the β-catenin (cadherin associated protein β 1, CTNN1) destruction complex as a means to enhance Wnt (wingless-type MMTV integration site family, member 1, WNT1) signaling that has been shown to promote memory stem cell accumulation and superior antitumor activity.[2]

The development of cancer vaccines is guided by measuring the induction immune responses during and shortly after vaccination, in effect assessing the magnitude of the vaccine-induced effector response. Given that a growing body of evidence suggests that persistence, rather than the initial magnitude, of the vaccine-induced immune response correlates with protective immunity, are we barking up the wrong tree? If yes, this study offers a clinically useful, cost-effective, and broadly applicable approach to promote the persistence of clinically efficacious vaccine-induced tumor immunity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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