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Pharmacologic Induction of CD8⁺ T Cell Memory: Better Living Through Chemistry

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Abstract

The generation of a robust population of memory T cells is critical for effective vaccine and cell-based therapies to prevent and treat infectious diseases and cancer. A series of recent papers have established a new, cell-intrinsic approach in which small molecules target key metabolic and developmental pathways to enhance the formation and maintenance of highly functional CD8⁺ memory T cells. These findings raise the exciting new possibility of using small molecules, many of which are already approved for human use, for the pharmacologic induction of immunologic memory.

INTRODUCTION

Immunologic memory is a hallmark of the adaptive immune system and provides for enhanced host protection by increasing the precursor frequency and functionality of antigen-specific B and T lymphocytes. CD8⁺ memory T cells, a subset of antigen-experienced T cells that recognizes intracellular-derived antigens, play a critical role in the immune response against cytosolic pathogens and cancers (1, 2). Previous strategies to enhance the generation of CD8⁺ T cell memory have focused on the modulation of the extent and quality of antigenic stimulation as well as the provision of co-stimulation, cytokines, and agonists of Toll-like receptors, a group of innate immune receptors that recognize structurally conserved microbial motifs (3). Each of these factors acts in a cell-extrinsic fashion to initiate receptor-mediated signaling cascades that ultimately result in increased memory formation and maintenance. Recently, we and others have reported in a series of papers a new approach to boost CD8⁺ T cell memory in a cell-intrinsic manner through the pharmacologic modulation of key metabolic and developmental pathways (4–6). In this Perspective, we highlight the intracellular pathways amenable to manipulation by small molecules that might enhance both the number and functionality of CD8⁺ memory T cells. We refer to this class of reagents as cell-intrinsic modulators of memory formation, or CIMMs, to distinguish them from classical adjuvants that function in a cell-extrinsic manner to enhance T cell function. To offer a framework for understanding how CIMMs might augment memory CD8⁺ T cell development, we begin by briefly discussing our current knowledge of the ontogeny of CD8⁺ T cell memory formation.

T CELL MEMORY FORMATION

During a primary immune response, naïve CD8⁺ T cells are recruited in specialized lymphoid organs and are specifically stimulated by pathogen- or tumor-associated antigens presented by professional antigen-presenting cells (APCs). Upon activation, specific T cells

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undergo a multi-log clonal expansion to generate large numbers of effector T cells capable of migrating to peripheral tissues in order to eliminate cells bearing the target antigen. Most of the responding population will undergo apoptosis, but a minority will persist as antigen-experienced memory T cells. CD8⁺ memory T cells are phenotypically and functionally diverse and have been broadly divided into two major subsets termed central memory T cells (T_{CM}s) and effector memory T cells (T_{EM}s), on the basis of their expression of the lymph node-homing molecules CD62L and CCR7 (7). T_{CM}s express high surface levels of CD62L and CCR7 and are capable of secreting interleukin (IL)-2, a key cytokine that modulates the expansion and the effector functions of lymphocytes, whereas T_{EM}s lack these lymph node-homing molecules, preferentially reside in the periphery, and are impaired in their ability to release IL-2. Although T_{EM}s can provide a first line of defense in peripheral sites of pathogen entry (8), such as the lung, skin, and mucosal surfaces, T_{CM}s might be more efficient than T_{EM}s in clearing systemic infections and treating tumors, as a result of their enhanced proliferative capacity and greater polyfunctionality (9, 10). More recently, a population of memory CD8⁺ T cells has been described that displays robust self-renewal and the multipotent capacity to derive T_{CM}, T_{EM}, and effector T cells after serial transplantation, prompting the label of memory stem cells (T_{SCM}s) (6, 11). Phenotypically, these cells exhibit a CD44^{low} CD62L^{high} phenotype like naïve T cells but co-express stem cell antigen-1 (SCA-1) as well as high levels of the antiapoptotic molecule B cell lymphoma 2 (Bcl-2) and the β chain of the IL-2 and IL-15 receptor CD122. The therapeutic potential of T_{SCM}s has just begun to be explored, but new findings indicate that this T cell subset might be more effective than other T cell memory subsets (6). Thus far, T_{SCM}s have been described in mice during allogeneic graft-versus-host responses or after activation of the Wnt signaling cascade (6, 11). Recently, Turtle and colleagues identified a human CD8⁺ memory T cell population that shares some phenotypic and functional characteristics with hematopoietic stem cells, such as the expression of the stem cell marker c-Kit and the ability to efflux cellular toxins through the ATP-binding cassette (ABC)-superfamily multidrug efflux protein ABCB1 (12). However, the prevalence of this population within the more differentiated T_{EM} subset makes them unlikely to represent the human counterpart of the T_{SCM}s described in mice, which phenotypically resemble naïve T cells.

The ontogeny of memory CD8⁺ T cells has been the subject of numerous investigations, but the lineage relationships between effector and memory subsets remain controversial (13). What is clear, however, is that CD8⁺ T cells at the peak of the primary response are heterogeneous with respect to their differentiation state and capacity to become memory T cells. Detailed analyses of the phenotype of responding CD8⁺ T cells have revealed that surface expression of the killer cell lectin-like receptor G1 (KLRG1) and the α chain of the receptor for IL-7 (IL-7R α) can distinguish CD8⁺ T cells fated to die or survive as long-lived memory cells (14, 15). Specifically, IL-7R α ^{high}KLRG1^{low} CD8⁺ T cells have a greater potential to persist and enter into the memory T cell pool, whereas IL-7R α ^{low}KLRG1^{high} CD8⁺ T cells represent terminally differentiated, senescent effector T cells destined to undergo apoptotic cell death.

The distribution of cells expressing these patterns of surface receptors is an integrated function of antigenic stimuli and inflammatory cues present during T cell priming. Cells receiving high-intensity signaling can undergo terminal effector differentiation mediated by the expression of the transcription factors T-bet (14) and eomesodermin (16), the antagonist of E-protein inhibitor of DNA binding 2 (Id2) (17), and the transcriptional repressor B lymphocyte-induced maturation protein 1 (Blimp1) (18). High-intensity signaling concurrently triggers reductions in the expression of pro-memory transcription factors such as Bcl-6 (19), Bcl-6b (20), T cell factor-1 (6), and the polycomb group member B lymphoma Mo-MLV insertion region 1 homolog (Bmi-1) (21). Thus, strong T cell activation can promote terminal differentiation at the expense of memory formation.

SHIFTING THE BALANCE TOWARD MEMORY WITH CIMMS

Enhancing fatty acid metabolism

The balance of these pro-differentiation and pro-memory factors in vaccinology has historically been tilted by using different antigen schedules, doses, and adjuvants (3), but it might now be possible to modulate these signals in a cell-intrinsic manner by targeting key pathways with CIMMs. Recently, Pearce and colleagues found that the activity of metabolic pathways involved in fatty and aliphatic amino acid metabolism controls the generation of CD8⁺ T cell memory independently of the magnitude of the effector response (5). Fatty acid oxidation (FAO) occurs within mitochondria and is regulated in part by adenosine monophosphate kinase (AMPK), a key sensor of cellular energy stores whose activity is promoted physiologically by the adenosine monophosphate:adenosine triphosphate (AMP:ATP) ratio and pharmacologically by the biguanide metformin (Fig. 1) (22). During an immune response, naïve T cells switch to anabolic glycolytic metabolism to provide the energy necessary to sustain high rates of proliferation and the acquisition of effector functions (23). As T cells transition from an activated effector state to a quiescent memory state, catabolic FAO is preferentially used for energy production. To facilitate this conversion, metformin was provided during the contraction phase, resulting in increased secondary responses and tumor prevention (5). Similarly, enhanced recall responses were observed with rapamycin, an inhibitor of the mammalian target of rapamycin complex 1 (mTORC1) historically used for its immunosuppressive properties to prevent the rejection of solid organ and allogeneic hematopoietic stem cell transplants (24).

Restraining mTOR-mediated protein translation

mTORC1 is a central metabolic node positively regulated by the activity of phosphoinositol 3-kinase (PI3K) and inhibited by the AMPK and glycogen synthase kinase-3 (GSK-3) pathways. Activation of mTORC1 promotes the translation of some mRNAs, such as those containing long and structured 5' untranslated region sequences via the p70 ribosomal protein S6 kinase 1 (p70S6K) and the eukaryotic translation initiation factor 4E (eIF4E) (Fig. 1) (24). These mRNAs often encode proteins involved in promoting cell growth and proliferation. Contemporaneously with Pearce, Araki and colleagues also found that rapamycin could enhance CD8⁺ T cell memory (4). In a detailed analysis, the authors found that the timing of drug administration was a critical determinant for the numbers, phenotype, and function of memory CD8⁺ T cells. Provision of rapamycin during the priming and expansion phase augmented the absolute numbers of memory T cells as a result of increased frequencies of IL-7Rα^{high} KLRG1^{low} CD8⁺ T cell memory precursors present at the peak of the responses. Conversely, administration during the contraction phase enhanced T_{CM} formation and secondary responses. Systemic administration of rapamycin can modulate many host populations, including professional APCs such as dendritic cells and suppressive T cell populations such as CD4⁺ forkhead box P3⁺ (FOXP3) regulatory T cells, in addition to the responding CD8⁺ T cells (24). Using retroviral transduction of short-hairpin RNAs targeting key components of the mTOR pathway into antigen-specific CD8⁺ T cells, Araki *et al.* could recapitulate the effect of rapamycin, demonstrating that the drug operated in a cell-intrinsic manner. Whether the primary effect of rapamycin is to enhance FAO as suggested by Pearce, or to restrain protein translation as shown by Araki, remains to be fully elucidated.

Inhibiting PI3K activity

An alternative pharmacologic approach for down-modulating mTORC1 activity might be the inhibition of PI3K, a pathway that transduces multiple pro-differentiation signals from the engagement of cytokine, co-stimulatory, and T cell receptors (Fig. 1) (24). PI3K inhibitors have yet to be explored as CIMMs, but recent findings indicate that these small

molecules can sustain the expression of the lymph node-homing markers CD62L and CCR7 that are associated with highly functional T_{CM}s (25). The PI3K inhibitor LY294002 inhibits the mTOR-mediated down-regulation of Krüppel-like factor-2 (Klf-2), a key transcriptional modulator for the expression of lymph node-homing receptors (25). PI3K inhibitors might also enhance CD8⁺ memory T cell formation by preventing PI3K/AKT-mediated cytosolic sequestration of forkhead box O1 (FOXO1), a transcription factor that controls the expression of Klf-2 and pro-memory factors such as BCL6 and IL-7Rα (26, 27). It should be noted that in addition to increasing FAO, metformin might also augment CD8⁺ T cell memory formation by increasing FOXO transcriptional activity through AMPK (27).

Activating the Wnt/β-catenin signaling pathway

Other than metabolic pathways, pharmacologic manipulation of key developmental pathways, such as Wnt, can potentially enhance the formation of CD8⁺ T cell memory (6). Wnt signaling negatively regulates the activity of GSK-3β, resulting in the accumulation and translocation of β-catenin into the nucleus, where it binds and activates the transcription factors lymphoid enhancer factor (LEF) and T cell factor (TCF) (Fig. 1). Wnt-mediated blockade of GSK-3β can also result in the activation of mTORC1 through the inhibition of the tuberous sclerosis complex 2 (TSC2) (28) (Fig. 1). Depending on the context, Wnt proteins can promote stem self-renewal and maintenance of pluripotency through their effects on β-catenin (29, 30), or they can induce proliferation, terminal differentiation, and senescence in systems where mTOR signaling is dominant (31, 32). Similar to what is observed in stem cell biology, we found that activation of Wnt signaling in CD8⁺ T cells through pharmacologic inhibition of GSK-3β facilitated the formation of self-renewing memory T cell subsets, including highly functional T_{SCMs}, by limiting proliferation and effector differentiation (6). These findings would suggest that GSK-3β inhibition in naïve CD8⁺ T cells preferentially enhances β-catenin signaling over its effect on mTORC1. How Wnt/β-catenin arrests effector differentiation and enhances CD8⁺ T_{SCM} formation remains to be completely elucidated, but TCF1 appears to play an important role because its overexpression alone can inhibit the acquisition of effector molecules such as interferon-γ (33).

TRANSLATING CIMMS INTO THE CLINIC

There is a critical need for reagents capable of enhancing preventative and therapeutic vaccines against human immunodeficiency virus, tuberculosis, viral hepatitis C, and cancer. Presently, alum is the only adjuvant approved by the U.S. Food and Drug Administration and it may be insufficient to trigger optimal T cell memory formation (34). Thus, CIMMs could rapidly increase the armamentarium of reagents capable of enhancing vaccines, because many candidate molecules are already approved or in clinical evaluation for other indications (Table 1). Some candidate CIMMs have established or putative immunosuppressive and antiproliferative activity, necessitating a careful evaluation of the dose. Araki and colleagues have demonstrated that, although low doses of rapamycin can potentiate CD8⁺ T cell memory formation, high doses can abrogate immune responses altogether (4). On the basis of mouse and nonhuman primate studies, we envision three modes of scheduling for CIMMs, correlating with different phases of the immune response (Fig. 2). The administration of CIMMs during the priming and expansion phase could inhibit terminal effector differentiation, resulting in increased frequencies of memory precursors, leading to greater absolute numbers of memory T cells (4, 6). Conversely, administration during the contraction phase minimally affects the absolute number of memory T cells but might cause an enrichment in the highly functional T_{CM} population (4, 5). The provision of CIMMs continuously during the immune response could have an additive effect and result in increased absolute numbers of memory T cells and T_{CM}s (4). The choice of the dosing schedule should be dictated by the safety profile of the CIMM

intended for use. For example, administration of inhibitors of GSK-3 β might be limited to the priming and expansion phase to avoid the oncogenic potential of chronic activation of the Wnt/ β -catenin pathway (29). Additionally, coadministration of different classes of CIMMs might be advantageous under certain circumstances. Because blockade of GSK-3 might have the undesirable downstream effect of promoting terminal differentiation by activating mTORC1, GSK-3 inhibitors might be more effectively used in combination with mTOR inhibitors such as rapamycin (32). Finally, we believe that proof-of-concept clinical trials should be performed in cancer patients with a high risk of recurrence, where a risk-to-benefit ratio is more favorable than in otherwise healthy patients. CIMMs might also be used in vitro to arrest terminal effector differentiation and generate memory-like CD8⁺ T cells, which have been shown to be the optimal population for cell-based therapies in animal models and early clinical trials (10, 35, 36). In summary, the modulation of key metabolic and developmental pathways by CIMMs appears to be a practical and translatable approach for the induction of enhanced CD8⁺ T cell memory.

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Fig. 1. Pharmacologic targets for the enhancement of CD8⁺ T cell memory formation

The activity of AMPK can be modulated by the AMP:ATP ratio or triggered by the biguanide metformin, resulting in inhibition of mTORC1 as well as augmentation of fatty acid oxidation and increased transcriptional activity of FOXO protein. FOXO protein acts in tandem with intranuclear binding partners to regulate gene transcription, but its binding partners involved in the modulation of pro-memory genes are incompletely elucidated. The PI3K-AKT pathway is activated by cytokine receptor engagement as well as T cell receptor and co-stimulatory receptor signaling (not shown) resulting in the downstream activation of mTORC1. PI3K-AKT activity can phosphorylate FOXO protein, promoting its cytosolic sequestration (not shown). PI3K can be inhibited by LY294002 or a number of small molecules which have recently entered clinical trials. In addition to indirect inhibition through modulation of upstream targets, mTORC1 may also be directly inhibited by rapamycin and its analogs. The activity of GSK-3 β inhibits mTORC1 through activation of TSC2 as well as targets β -catenin for proteasomal degradation. Inhibition of GSK-3 β using TWS119 mimics Wnt signaling by promoting β -catenin accumulation and translocation into the nucleus, where it binds to partners including LEF and TCF to promote gene transcription. PTEN, phosphatase and tensin homolog; PDK1, pyruvate dehydrogenase kinase 1; Rheb, Ras homolog enriched in brain; G β L, G protein beta subunit-like; 4E-BP1,

phosphorylated heat- and acid-stable protein regulated by insulin 1; p70S6K, p70 ribosomal protein S6 kinase 1; APC, adenomatous polyposis coli; CK1 α , casein kinase 1, alpha 1; Dvl, disheveled; LRP5/6, low-density lipoprotein receptor-related protein 5/6.

Fig. 2. The timing of CIMM administration affects both the phenotype and numbers of CD8⁺ memory T cells

(A) Kinetics of a prototypical primary T cell immune response. After antigenic stimulation, naïve CD8⁺ T cells undergo a multi-log clonal expansion, peaking during the first week. The majority of CD8⁺ T cells become terminally differentiated effector cells that rapidly undergo apoptosis. A minority of responding T cells (T_{CM} and T_{EM} precursors) will persist as long-lived memory T cells. (B) Effect of CIMM administration during the priming and expansion phase of an immune response. Provision of CIMM during the first week of a primary immune response minimally affects the total number of responding T cells at the peak of the response but yields greater numbers of memory T cell precursors, leading to increased

numbers of memory T cells without altering the relative distribution of T_{CM} s and T_{EM} s. To date, the effect of metformin has yet to be directly tested during this phase. (C) Effect of CIMM administration during the contraction phase of an immune response. The provision of CIMMs after the peak of an immune response results in a more rapid acquisition of the phenotypic and functional attributes associated with central memory T cells, without changing the absolute number of persisting memory T cells. To date, the GSK-3 β inhibitor TWS119 has not been tested during this phase. Curves are extrapolated from Araki *et al.* (4).

Table 1
Clinical development and current indications of candidate CIMMs

Data were obtained from ongoing or completed human clinical trials listed at <http://www.clinicaltrials.gov>.

Metabolic pathway	Molecular target	Stage of clinical development; indication
mTOR		
Rapamycin	mTORC1	Approved; prophylaxis of organ rejection
Temsirolimus	mTORC1	Approved; advanced renal cell carcinoma
Everolimus	mTORC1	Approved; advanced renal cell carcinoma
Ridaforolimus	mTORC1	Phase II; advanced solid and hematologic malignancies
OSI-027	mTORC1 and mTORC2	Phase I; advanced solid and hematologic malignancies
Oleandrin	p70S6K	Phase I; advanced solid tumors
AMPK		
Metformin	AMPK	Approved; type II diabetes
AICAR	AMPK	Phase II; type II diabetes, Lesch-Nyhan syndrome
GW501516	AMPK	Phase II; dyslipidemia
PI3K		
SF1126 (LY294002 pro-drug)	PI3K	Phase I; advanced solid tumors
CAL-101	PI3K (p110 δ)	Phase I; hematologic malignancies
PX-866	PI3K (p110 α)	Phase I; advanced solid tumors
MK-2206	AKT	Phase I; advanced solid tumors
Oleandrin	AKT	Phase I; advanced solid tumors
Wnt/β-catenin		
Lithium	GSK-3 α and - β	Approved; manic-depressive disorder
Valproic acid	GSK-3 β	Approved; seizure disorder
Flavopiridol	GSK-3 α and - β	Phase II; advanced solid and hematologic malignancies
Staurosporine	GSK-3 α and - β	Phase II; advanced solid and hematologic malignancies
NP031112	GSK-3 β	Phase I/II; Alzheimer's disease
TWS119	GSK-3 β	Preclinical