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The mTOR Kinase Determines Effector versus Memory CD8$^+$ T Cell Fate by Regulating the Expression of Transcription Factors T-bet and Eomesodermin

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SUMMARY

The mechanisms underpinning integration of instructions that program naive CD8$^+$ T cells for effector and/or memory differentiation are not well understood. Herein, we demonstrate that interleukin-12 (IL-12) enhanced and sustained antigen and costimulatory molecule (B7.1)-induced mTOR kinase activity in naive CD8$^+$ (OT-I) T cells via phosphoinositide 3-kinase and STAT4 transcription factor pathways. Blocking mTOR activity by rapamycin reversed IL-12-induced effector functions because of loss of persistent expression of the transcription factor T-bet. Rapamycin treatment of IL-12-conditioned OT-I cells promoted persistent Eomesodermin expression and produced memory cell precursors that demonstrated enhanced sustenance and antigen-recall responses upon adoptive transfer. The memory cell precursors showed greater tumor efficacy than IL-12-conditioned effector OT-I cells. These results identify mTOR as the central regulator of transcriptional programs that determine effector and/or memory cell fates in CD8$^+$ T cells. Targeting mTOR activity offers new opportunities to regulate CD8$^+$ T cell-mediated immunity.

INTRODUCTION

The nature and intensity of instructions received by a naive CD8$^+$ T cell orchestrates gene programs that guide their differentiation into various functional subsets (Iezzi et al., 1998; Joshi et al., 2007). The presence of cytokines during antigen stimulation is instrumental in regulating the transcriptional program of CD8$^+$ T cells for effector and memory functions (Curtsinger et al., 2003; Xiao et al., 2009). However, the molecular mechanisms by which integration of cytokine-generated signals determine antigen- and costimulation-induced T cell responses are not well defined. Based on the type of cytokine present during antigen stimulation, naive T cells could give rise to either type I (interferon-γ [IFN-γ]), type II (interleukin-4 [IL-4]), type 17 (interleukin-17 [IL-17]), or regulatory (transforming growth factor-β [TGF-β], interleukin-10 [IL-10]) functional outcomes, but the instructional requirements and the transcriptional regulators for CD8$^+$ T cells are poorly defined and the understanding is based on information generated by the use of CD4$^+$ T cells (Bettelli et al., 2007; Zhou et al., 2009). Besides the ability of effector CD8$^+$ T cells to protect against variety of challenges, their ability to produce memory functions is crucial for host immunity. Emerging insights into CD8$^+$ T cell memory generation demonstrates the variety of pathways that can give rise to memory cells (Kaech and Wherry, 2007; Lefrançois and Marzo, 2006). Moreover, the cell type and the pathway used for the emergent memory cells may impart functional diversity to the memory CD8$^+$ T cells, thus emphasizing the role played by instructions early during antigen stimulation for not only effector differentiation but also for memory functions.

Several transcription factors have been shown to coordinate and regulate the balance between long-lived memory and terminally differentiated effector CD8$^+$ T cells (Intlekofer et al., 2005; Joshi et al., 2007; Welsh, 2009). The transcription factor T-bet (*Tbx21*) is the master regulator of type I effector differentiation whose expression is considerably enhanced and sustained in the presence of IL-12 (Joshi et al., 2007; Szabo et al., 2000). Recent evidence suggests that inflammation-induced T-bet can control effector and memory fate decisions in CD8$^+$ T cells because increased T-bet expression promotes short-lived effector cells with a KLRG1^{hi} and IL-7R^{lo} phenotype, whereas low T-bet expression promotes long-lived memory cells (Joshi et al., 2007). Eomesodermin (*Eomes*), another T-box containing transcription factor, whose expression increases from the effector to memory phases of an immune response, is proposed to promote memory formation (Intlekofer et al., 2005). Moreover, IL-12 induces T-bet but inhibits Eomesodermin expression to favor effector versus memory generation (Takemoto et al., 2006), suggesting the importance of understanding cell-intrinsic factors that regulate T-bet and Eomesodermin expression which may enable achieving desirable CD8$^+$ T cell functional outcomes.

The energy-sensitive kinase mammalian target of rapamycin (mTOR) has the ability to sense cellular metabolic state (ATP:AMP), extracellular nutrient availability, presence of growth factors/cytokines, and control key cellular processes, including ribosomal genesis, apoptosis/autophagy, proliferation, and cell growth that govern cell fate (Dennis et al., 2001; Wullschleger et al., 2006). The immunosuppressive drug rapamycin (specific

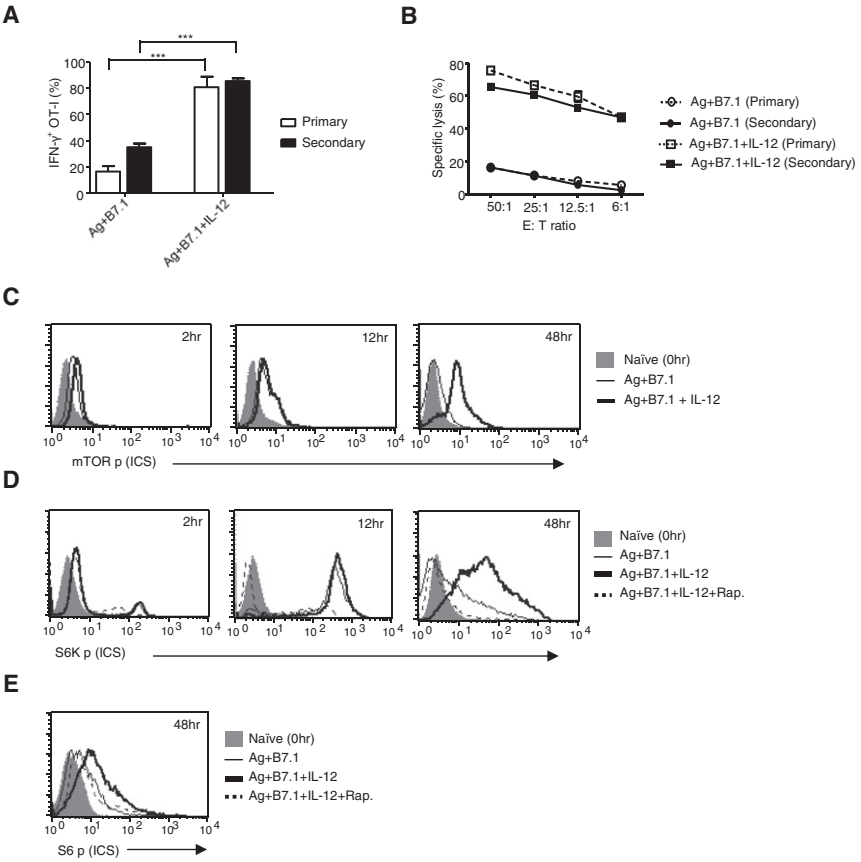

Figure 1. Instructions that Program Naive CD8$^+$ T Cell for Type I Effector Maturation Enhances and Sustains mTOR Activity

(A and B) OT-I cells stimulated with BOK (Ag+B7.1) (±) IL-12 were evaluated for (A) IFN-γ by ICS and (B) cytolytic activity (primary, 72 hr poststimulation; secondary, 24 hr postsecondary stimulation); ***$p < 0.0002$.

(C–E) OT-I cells stimulated with antigen (Ag) (SIINFEKL, 10 nM) plus B7.1 (100 μg/ml) (Ag+B7.1) (±) IL-12 (2 ng/ml) were evaluated by ICS at the indicated time points for (C) phosphorylated mTOR, (D) phosphorylated S6K, and (E) phosphorylated ribosomal S6. For mTOR inhibition, rapamycin (20 ng/ml) was added 30 min prior to addition of antigen, cytokine. Data are representative of at least three independent experiments with similar outcomes. (Data are presented as mean ± SEM.) See also Figure S1.

inhibitor of mTOR) is widely used to restrict allograft rejection reactions (Saunders et al., 2001), and most mechanistic studies to date have been focused on understanding the action of rapamycin on CD4$^+$ T cell responses. These studies have demonstrated that mTOR inhibition by rapamycin induces CD4$^+$ T cell anergy and/or differentiation into (FoxP3$^+$) regulatory T cells (Kang et al., 2008; Zheng et al., 2007). Moreover, a recent report has implicated the requirement of mTOR kinase signaling in regulating effector versus regulatory cell lineage commitment in CD4$^+$ T cells (Delgoffe et al., 2009). Interestingly, recent reports have demonstrated the role of mTOR in regulating CD8$^+$ T cell trafficking (Sinclair et al., 2008) and memory differentiation (Araki et al., 2009; Pearce et al., 2009), but the molecular mechanisms by which mTOR regulates CD8$^+$ T cell differentiation or trafficking remain uncharacterized.

In this study, we have identified the role of mTOR in instructional programming of naive CD8$^+$ T cells for effector and/or memory fate by regulating expression of T-bet and Eomesodermin. Inhibition of mTOR activity blocked persistent T-bet expression and promoted memory-precursor generation that showed greater tumor efficacy than type I effector CD8$^+$ T cells. Thus, our studies suggest a model in which mTOR is a rheostat, which depending upon the nature and intensity of signals received, regulates the transcriptional balance to control CD8$^+$ T cell effector function and/or memory generation.

RESULTS

Instructions that Program Naive CD8$^+$ T Cell for Type I Effector Differentiation Augment mTOR Activity

To characterize mechanisms underpinning instructional (signals 1, 2, and 3—antigen [Ag], B7.1 [costimulation], and IL-12 [cytokine], respectively) programming of naive CD8$^+$ T cells for type I effector functions, we initiated our studies to confirm the deterministic role of IL-12 in imparting type I effector maturation in OT-I cells stimulated with adherent cell line, namely BOK expressing H-2K^b, OVAp (SIINFEKL), and B7.1. As anticipated, addition of IL-12 resulted in robust IFN-γ production and cytotoxic T lymphocyte (CTL) activity in OT-I cells at 72 hr (Figures 1A and 1B; primary). Furthermore, when the primary effector OT-I cells (72 hr) were rested with IL-7 for an additional 72 hr (12% IFN-γ detected at 144 hr) and restimulated with Ag and B7.1 (see Experimental Procedures), only the IL-12-conditioned OT-I cells reinduced IFN-γ and CTL activity (Figures 1A and 1B; secondary). Thus, IL-12 has a deterministic role in CD8$^+$ T cell effector maturation.

Although the kinase mTOR has been implicated as an integrator of various extracellular signals and a sensor for internal energy levels for determination of cell fate (Hay and Sonenberg, 2004), the role for mTOR in integrating instructions that program naive CD8$^+$ T cells for type I effector differentiation is unclear. First, we tested the ability of Ag and B7.1 (Ag+B7.1) in the presence or absence of IL-12 to activate mTOR in OT-I cells at various time points after stimulation. Stimulation of naive OT-I cells with Ag+B7.1 induced mTOR phosphorylation (activation) by 2 hr, which was maximal at 12 hr and barely detectable by 48 hr (Figure 1C). Remarkably, IL-12 addition enhanced Ag+B7.1-induced mTOR phosphorylation at 2 hr, which was maintained at 48 hr after stimulation (Figure 1C). Thus, although Ag+B7.1 induces mTOR phosphorylation, the