Published in final edited form as:

Clin Immunol. 2012 February ; 142(2): 95–96. doi:10.1016/j.clim.2011.10.004.

Targeted immunosuppression: No longer naïve

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Naïve T cells patrol the body in search of their cognate antigen. However, it has become better appreciated that the outcome of antigen recognition is increasingly complex. That is, it is not merely a question of whether the T cell will become activated, shut off, or deleted. Rather, there are multiple effector and regulatory T cell fates that a T cell may take, and this fate decision is profoundly influenced by the environmental signals that are encountered concomitant with TCR engagement. The mTOR pathway is emerging as a critical integrator of environmental cues that direct T cell fate. For CD4⁺ T cells, mTOR has been shown to influence Th1, Th2, Th17 and T regulatory cell skewing [1,2]. For CD8⁺ T cells, mTOR has been implicated in memory cell differentiation [3,4]. These diverse fates are enforced by differential gene expression, DNA methylation, and chromatin structure as well as differential signaling pathways. In light of this complexity it would seem rather naïve to assume that naïve T cells and effector T cells would behave similarly to pharmacologic targeting of signal transduction pathways. In this issue of CLIM, Lin et al. demonstrate precisely this point [5]. These investigators examine the effects of PI3 kinase (PI3K) and mTOR inhibitors on T cells and find that previously activated (experienced) T cells become resistant to inhibition of a single pathway but are sensitive to dual inhibition. Combining the mTOR-inhibiting effects of rapamycin with small molecules that target different steps in the PI3K/ERK/RSK pathway potently suppresses the activation of experienced T cells *in vitro* and slows the onset of collagen-induced arthritis in mice. A critical target of these pathways is the ribosomal translational machinery, and Lin et al. propose that targeting translation in T cells at multiple points may be the best way to restrain the activity of experienced T cells.

There are multiple pathways that drive ribosomal activity in T cells. T cell activation leads to the activation of the mTOR/Raptor (or TORC1) complex that directly phosphorylates the p70 ribosomal S6 kinase (S6K1 or P70S6K) and directly inhibits the translational repressor 4EBP1 [6,7]. Costimulatory receptor engagement and cytokine receptor activation activate PI3K activity toward both protein and lipid targets, resulting in activation of Akt and repression of TSC2, further driving TORC1 activity [8]. Receptor tyrosine kinase activity (such as the IL-2 receptor, which also activates mTOR) drives the Ras/Raf pathway, resulting in activity of MEK1/2, their targets, ERK1/2, and ultimately phosphorylation and activation of the p90 ribosomal kinase (RSK or P90RSK) [9]. Both RSK and S6K1 phosphorylate and activate the ribosomal subunit S6, which is essential for translation. Though the PI3K, mTOR, and MEK/ERK pathways affect many cellular outputs, they overlap in their control of protein translation; indeed, ERK1/2 can also phosphorylate S6K1

under some circumstances. Both S6K1 and RSK target S6, and the authors reasoned that this redundant activation of S6 might lead to synergy between mTOR and PI3K inhibition.

A critical event in the activation of T cells is the upregulation of IL-2 expression; autocrine IL-2 signaling drives the initial wave of CD4 proliferation and maturation after TCR stimulation. Lin et al. examine the different requirements of naïve and experienced CD4 cells for activation after TCR stimulation. They show that experienced CD4 cells no longer require IL-2 signaling in order to proliferate after TCR stimulation. Inhibition of either mTOR by rapamycin or PI3K by LY249002 in naïve CD4 cells blocks phosphorylation of S6K1 and S6 and prevents phosphorylation. In comparison, experienced CD4 cells appear resistant to rapamycin and continue to proliferate and phosphorylate S6 after TCR stimulation, and they are only partly sensitive to LY249002. Experienced CD4 cells treated with rapamycin and inhibitors of either MEK1/2 or RSK lose their proliferative capacity and do not efficiently phosphorylate S6. In light of these results, the authors next examined the effect of dual mTOR/ERK inhibition *in vivo* in a mouse model of collagen-induced arthritis. Rapamycin and the MEK inhibitor PD325901 administered either before or concomitant with collagen slowed the onset of joint disease, and there was an additive effect in mice treated with both inhibitors. Strikingly, dual inhibition appeared to block the earliest onset of inflammation, but joint histology at 10 days showed no significant difference between dual inhibition and single inhibition with rapamycin or PD325901. When the authors examined phosphorylation of S6 as marker of translation activity in collagen-stimulated CD4 cells from collagen-treated mice, dual inhibition restored phospho-S6 levels to those seen in unstimulated cells.

Interestingly, after an initial phase of *in vitro* stimulation, CD4 T cells become resistant to rapamycin (and partly resistant to LY249002). How does this resistance arise? The TORC1 complex (mTOR, Raptor, and mLST8) directs the phosphorylation of S6K1 and is typically more sensitive to inhibition by rapamycin than the Rictor-containing TORC2 complex. However, genetic and proteomic studies have revealed that a subset of bona fide TORC1 substrates remain largely rapamycin-insensitive [10,11]. Many mechanisms of rapamycin resistance have been proposed, including the relative levels of FKBP12 and TORC components in a cell, the competitive binding of phosphatidic acid to FKBP12, and the post-translational modification of TORC components [12]. Lin et al. show that at least some elements of rapamycin sensitivity are reversed by co-inhibition of RSK, which phosphorylates S6. Just as the threshold for TCR signaling is lowered after a naïve CD4 cell's first encounter with antigen and costimulation, so the threshold for translational activation downstream of mTOR is lowered. The molecular changes that accompany this remain to be identified.

Because multiple pathways converge to activate translation, it is no surprise that inhibiting multiple steps is more potent than single inhibition. These findings have important clinical implications as several new classes of inhibitors near the clinic. ATP-competitive mTOR kinase inhibitors such as Torin1 and pp242 efficiently inhibit both TORC1 and another mTOR complex, TORC2, which drives Akt activation. This compound class has attracted intense interest in the oncology world, and three class members (AZ-8055, OSI-027, and INK128) have entered clinical trials [13]. MEK inhibitors are currently in clinical trials for activity against melanoma and hepatocellular carcinoma [14,15]. Also, because mTOR and PI3K share sequence homology in the hinge region of their kinase domains, several compounds developed as potent PI3K inhibitors were serendipitously found to block mTOR activity as well. These compounds, such as NVP-BEZ235 and PI-103, have also begun clinical trials for solid cancers such as ovarian and breast; these compounds make the dual-hit therapy envisioned by Lin et al. a single-agent possibility. Furthermore, as rapalogs, mTOR and PI3K kinase inhibitors, and MEK inhibitors all inhibit translation by different

mechanisms it may be possible to circumvent or even prevent resistance through the combination of different agents.

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