RESEARCH HIGHLIGHT

T cell priming: let there be light

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Activation of naïve T cells via the T cell receptor (TCR) induces proliferation, gain of effector functions, and ultimately the development of long-lived memory cells. Memory cells have lower thresholds of activation than naïve cells and respond more robustly to similar degrees of stimulation, which are fundamental properties of adaptive immunity. TCR occupancy leads to phosphorylation of TCR- ζ and CD3 cytoplasmic tails by Lck and Fyn, recruitment of ζ -associated protein kinase 70 (ZAP70), and phosphorylation/activation of downstream targets such as the linker for activation of T cells (LAT) and SLP-76 [1]. Phosphorylated LAT binds to the PLC- γ 1 N-terminal SH2 domain and recruits the molecule to the plasma membrane, where it is phosphorylated on activating residues and cleaves phosphatidylinositol 4,5-bisphosphate to yield two biologically active products: 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). IP₃ is necessary for triggering Ca²⁺ flux and activation of critical transcription factors such as nuclear factor of activated T cells (NF-ATc) and DAG upregulates the activity of protein kinase C and contributes to Ras and mitogen-activated protein kinase (MAPK) cascade activation [1]. How changes in these proximal signaling events might account for the

sensitive and rapid response of antigen-experienced cells has been an area of much study, and among the possibilities there are data indicating that such cells have increased basal phosphorylation, downregulation of phosphatases, and qualitative changes in the association of signaling molecules with membrane lipid rafts [2].

The question of why naïve and previously activated human T cells differ in their activation responses has been revisited in a recent paper by von Essen and colleagues [3], who come to the unexpected conclusion that a chain of events involving p38 MAP kinase (p38 MAPK), vitamin D3 receptor (VDR), and PLC- γ 1, is responsible. In that paper "primed" T cells were generated by culturing naïve T cells in the presence of anti-CD3/anti-CD28 plus IL-2 for up to 9 days, and then analyzed for their responses after a 24 h or longer rest in the absence of stimuli. Certain TCR-proximal signaling events (ZAP70 and LAT phosphorylation) were actually enhanced in naïve compared to primed T cells, whereas others (Ca²⁺ flux and ERK activation) were diminished. The latter was explained by the finding that PLC- γ 1 was expressed at very low levels in naïve T cells, but increased up to 80-fold during the course of *in vitro* priming. Because of previous observations that vitamin D can upregulate PLC- γ 1 in some cells, and that VDR levels in human T cell are upregulated by activation, a role for this receptor/

hormone pair was investigated. Indeed, activation-induced VDR upregulation preceded PLC- γ 1 upregulation, and the latter was inhibited by VDR antagonists or an inhibitor of intracellular conversion of the inactive vitamin D precursor to the active metabolite, 1,25(OH)₂D₃. If VDR upregulation precedes PLC- γ 1/Ca²⁺/ERK, what TCR proximal signal is responsible for its expression? The answer seems to be p38, which in T cells can be activated via a pathway distinct from the classic MAPK cascade. Stimulation via the TCR leads to ZAP-70-dependent phosphorylation of p38 on Tyr-323, resulting in autophosphorylation of the activation loop of the kinase [4]. Studies using "knockin" mice in which p38 α Tyr-323 was replaced with a Phe have shown that this alternative pathway is indispensable for TCR-induced, but not stress-induced, p38 activation in T cells, and in its absence there is a delay in the onset of proliferation and reduced IFN- γ expression [5]. von Essen *et al.* found that p38 activation was actually enhanced in naïve compared to primed T cells and was inhibited (along with VDR upregulation) by inhibition of p38 kinase activity, a hallmark of the alternative p38 activation pathway because it prevents the necessary autophosphorylation step. The data (summarized schematically in Figure 1) describe a previously unappreciated bottleneck in naïve human T cell activation, and lead to the notable conclusion that 1,25(OH)₂D₃ is an es-

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Figure 1 Overview of pertinent differences in TCR signaling between naïve and primed human T cells. Phosphorylation of ZAP70 and LAT is robust in naïve but not primed T cells; the reciprocal is true for Ca²⁺ flux and ERK activation. In naïve T cells, TCR engagement leads to activation of p38 via its phosphorylation on Tyr-323 by ZAP70 (the alternative pathway), resulting in upregulation of VDR and then PLC-γ1. Primed T cells express PLC-γ1, which is recruited to phosphorylated LAT, activated, and cleaves phosphatidylinositol 4,5-bisphosphate to yield IP₃ and subsequent Ca²⁺ flux.

essential cofactor in T cell activation and expansion. Indeed, the authors found that *in vitro* proliferation of T cells from patients with vitamin D-deficiency was diminished compared to healthy controls, but was restored by the addition of 1,25(OH)₂D₃ to the cultures.

The notion that vitamin D has a positive effect on immune defense has been based in part on epidemiological data linking seasonal changes in vitamin D serum levels with seasonal peaks of upper respiratory infections (URI), which led to studies using ultraviolet radiation or administration of cod liver oil (which contains vitamin D) that showed a reduction in the incidence of URI [6]. There is also evidence for an inverse correlation between URI incidence and 1,25(OH)₂D₃ serum levels, regardless of season [7]. Vitamin D has been also shown to have effects on the innate immune response, inhibiting proliferation of *Mycobacterium tuberculosis* in macrophages [6], and the majority of *M. tuberculosis* patients has low levels

of serum vitamin D, supplementation of which results in greater clinical improvement than standard therapy alone [8]. Other studies have suggested a link between vitamin D insufficiency and autoimmune diseases such as multiple sclerosis and Crohn's disease [6] which may possibly be because vitamin D3 enhances Th2 differentiation at the expense of Th1 cells [9].

Most of the *in vitro* work on vitamin D3 and immunity has focused on mixed cell populations or cells of the innate immune system. Those studies that examined the direct effect of vitamin D3 on T cells generally found it to be inhibitory for pro-inflammatory Th1 cytokine production. For example, IL-2 production by TCR-mediated stimulation of murine T cell hybridomas, fusions between activated T cells and an immortalized thymoma, was blunted by a presence of vitamin D3 [10]. Studies with primary mouse or human CD4⁺ T cells found that activation in the presence of 1,25(OH)₂D₃ inhibited T cell

pro-inflammatory cytokine production and "help" for B cell immunoglobulin secretion, at the same time causing an increase of CTLA-4 and Foxp3 and a shift toward Th2-regulatory transcription factors and Th2 cytokines [6, 9, 11]. Effects on T cell proliferation were variable, ranging from negligible to modest inhibition. Taken together with the present report, it appears that vitamin D3 intersects with T cell activation at both proximal (PLC-γ1 upregulation) and distal (transcription factor and cytokine production) points. One reason that the importance of vitamin D in human T cell priming has not been previously appreciated may be that naïve murine and human T cells differ, the former already expressing VDR and PLC-γ1 [12].

One of the intriguing aspects of the present paper is that the alternative p38 activation pathway was critical in the upregulation of VDR, revealing a previously unknown connection between TCR-induced p38 activation and VDR-PLC-γ1 signaling. The ZAP-70-depen-

dent alternative activation of p38 has been observed only in TCR-stimulated T cells. Unlike p38 activated via classic MAP kinase signaling cascade, which is phosphorylated on a Thr and Tyr residue, p38 activated by the alternative pathway is predominantly monophosphorylated on Thr180 [13]. Moreover, the mono- and dual-phosphorylated p38 species have been shown to have distinct substrate fine-specificities, raising the possibility that the alternatively activated p38 might serve unique functions in T cells. It would be interesting, therefore, to ask if activation of T cell p38 via the MAPK cascade would have the same positive effect on VDR upregulation as the alternative pathway in human T cells.

There has been a growing appreciation that nuclear hormone receptors play important roles in T cell growth, differentiation, and function [14, 15]. The present paper adds 1,25(OH)₂D₃ to this list and provides a rationale for exploring its possible use in dealing with infections in patient populations that might be deficient in this vitamin.

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