DdH

TOLERANCE

PD1 — a multitasking receptor

“PD1 exerts its inhibitory effects on T cells by regulating TCR signalling through both IL-2-dependent and -independent mechanisms”

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Self-reactive T cells that have escaped central tolerance are controlled through a process termed peripheral tolerance, and evidence indicates that the receptor PD1 (programmed cell death protein 1) is an essential player in this process. Now, a study by Patsoukis *et al.* provides new insights into the many mechanisms by which PD1 inhibits T cell proliferation.

When primary human CD4⁺ T cells that had been activated through the T cell receptor (TCR)–CD3 complex and CD28 were treated with an agonistic antibody specific for PD1, the cells arrested at the G1 phase of the cell cycle. As cell cycle progression is tightly regulated by cyclins, cyclin-dependent kinases (CDKs) and CDK inhibitors, the researchers focused on the effect of PD1 on this cell cycle machinery. They showed that PD1 signalling did not prevent the production of G1 phase-specific cyclin D or the expression of late G1 phase-specific cyclin E and associated CDK2, but did affect the activation of CDK2 and its inhibitor p27, which functions at the G1–S phase transition. The degradation of p27 is mediated by the ubiquitin ligase SCF^{SKP2} (a SKP1–cullin 1–F-box protein complex in which the F-box protein is SKP2), and the researchers showed that PD1 signalling prevented increases in SKP2 levels in response to TCR stimulation. This led to the accumulation of p27, inhibition of CDK2 activity and a block on G1–S phase transition.

CDK2 also phosphorylates the signal transducer SMAD3, reducing both the ability of SMAD3 to inhibit G1–S phase progression and its transcriptional activity. Patsoukis and co-workers found that PD1 signalling decreased SMAD3 phosphorylation and increased its transcriptional activity. p15^{INK4B}, an inhibitor of CDK4 and CDK6, is an important transcriptional target of SMAD3. Moreover, SMAD3 transcriptionally represses the

CDK activator CDC25A. In activated T cells, PD1 signalling not only increased the expression of the gene encoding p15^{INK4B} and decreased the levels of CDC25A mRNA, but also increased the degradation of CDC25A, resulting in reduced activity levels of CDK4 and CDK6 and therefore G1 arrest. When SMAD3 expression was knocked down, PD1 signalling no longer increased the levels of p15^{INK4B}, indicating that SMAD3 is a crucial downstream component in the PD1-mediated G1 phase arrest of activated T cells.

But how does PD1 regulate SKP2 expression? PD1 is known to inhibit TCR-mediated activation of the PI3K–AKT pathway, which is required for SKP2 expression. The researchers found that PD1 also inhibits the activation of the MEK–ERK signalling pathway — which is also required for the expression of SKP2 — via the inhibition of the upstream signalling molecule RAS.

Finally, Patsoukis *et al.* showed that adding exogenous interleukin-2 (IL-2), a proliferation-promoting cytokine, to the T cell culture did not affect AKT activation but partially re-established the MEK–ERK pathway. This partially restored SKP2 expression and increased T cell proliferation, indicating that PD1 exerts its inhibitory effects on T cells by regulating TCR signalling through both IL-2-dependent and -independent mechanisms.

To sum up, PD1 signalling targets multiple regulators of the cell cycle to block cell cycle progression and the proliferation of T cells, explaining its key role in the induction and maintenance of anergy and peripheral tolerance.

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ORIGINAL RESEARCH PAPER Patsoukis, N. *et al.* Selective effects of PD-1 on Akt and Ras pathways regulate molecular components of the cell cycle and inhibit T cell proliferation. *Sci. Signal.* **5**, ra46 (2012)