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T CELLS

Successful checkpoint blockade requires positive co-stimulation

T cell activity is controlled by a combination of antigen-specific signals from the T cell receptor (TCR) and antigen-independent signals from co-receptors. Therapeutic approaches that target co-inhibitory ('checkpoint') receptor–ligand pairs such as PD1–PDL1 have shown clinical activity in many different types of cancer and promise for the treatment of chronic viral infections. However, the clinical development of checkpoint inhibitors (particularly for cancer) has far-outpaced the molecular understanding of how they work, and why responses are seen in only some patients. Now, two papers in *Science* demonstrate that the co-stimulatory receptor CD28 has a central role in PD1 signalling and the success of PD1-targeted therapies.

> "PD1 inhibits T cell activation by suppressing positive co-stimulation through CD28"

Mellman, Vale and colleagues investigated the molecular mechanisms of PD1 signalling in a cell-free membrane reconstitution system containing 11 different proteins. They found that the cytoplasmic domain of PD1 was predominantly phosphorylated by the tyrosine kinase Lck. This was followed by the recruitment of the tyrosine phosphatase Shp2, which exhibited exquisite specificity for phosphorylated PD1. It had previously been assumed that PD1 signalling suppresses T cell activation by dephosphorylating the TCR or TCR-associated molecules. However, CD28 and — to a lesser extent — Lck proved to be the most sensitive targets for dephosphorylation by the PD1–Shp2 complex.

In antigen-stimulated $CD8^+$ T cells, CD28 and PD1 were found to co-migrate, eventually collapsing into a bull's-eye pattern around a central TCR island. To test whether PD1 signalling also induces CD28 dephosphorylation in live cells, Jurkat T cells were stimulated with antigen-loaded Raji B cells, a system widely used for studying TCR and CD28 signalling. Jurkat and Raji cells were engineered to express defined amounts of PD1 and PDL1 respectively, and $PD1^+$ Jurkat cells were then stimulated with different ratios of $PDL1^{hi}$ to $PDL1^-$ Raji cells. CD28 phosphorylation in Jurkat cells was found to decrease as a function of the percentage of $PDL1^{hi}$ cells encountered, whereas phosphorylation levels of the TCR and its downstream components were not significantly affected by PD1 signalling. These results indicate that PD1 inhibits T cell activation by suppressing co-stimulation through CD28 in a cell-intrinsic manner.

Meanwhile, Rafi Ahmed and colleagues studied the role of CD28 and its ligand B7 in the context of PD1-targeted therapy *in vivo*, using mouse models of chronic viral infection (LMCV) and cancer (colon carcinoma). Inhibition of CD28 signalling in the LCMV model (using CTLA4-Ig, B7-blocking antibodies or genetic deletion of CD28) abrogated the capacity of PDL1-targeted antibodies to rescue exhausted $CD8^+$ T cells. In the colon carcinoma model, PDL1-targeted therapy elicited $CD8^+$ T cell-mediated tumour regression in 8 out of 9 animals, whereas 8 out of 10 animals that were treated with a combination of PDL1-specific and B7-specific blocking antibodies showed tumour progression.

In humans, $CD8^+$ T cells can lose CD28 expression, and the authors found variable CD28 expression in tumour infiltrating lymphocytes (TILs) from patients with non-small cell lung cancer (NSCLC). However, in NSCLC patients who had received PD1-targeted therapy, proliferating $CD8^+$ T cells in the peripheral blood were mostly $CD28^+$, implying a selective expansion of these cells.

Although many studies have focused on the expression of inhibitory receptors by TILs, positive co-stimulation has not been a major focus. These results indicate that CD28 co-stimulation is necessary for effective PD1-directed therapy, and suggest that CD28 expression on TILs may serve as a potential biomarker to predict responsiveness to treatment.

Alexandra Flemming

ORIGINAL ARTICLES Kamphorst, A. O. *et al.* Rescue of exhausted CD8 T cells by PD-1-targeted therapies is CD28-dependent. *Science* http://dx.doi.org/10.1126/science.aaf0683 (2017) | *Hui, E. et al.* T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science* http://dx.doi.org/10.1126/science.aaf1292 (2017)