

Dario A.A. Vignali

Molecular control of T cell development and function

Dario A.A. Vignali PhD. Vice Chair and Member.

Department of Immunology, St. Jude Children's Research Hospital, Memphis, USA.

T cell fate in the thymus is determined by the strength of signal through the T cell receptor (TCR):CD3 complex that is received upon engagement with self-peptide:MHC complexes. The TCR has specialized cytoplasmic motifs (ITAMs) that mediates signal transduction upon tyrosine phosphorylation. The TCR:CD3 complex is unique in having ten ITAMs but the physiological significance of this is unclear. We have generated 25 groups of mice expressing different combinations of wild type and ITAM mutant TCR:CD3 complexes using multicistronic retroviral vectors and retroviral-mediated stem cell gene transfer. We show that mice with less than 7 wild type CD3 ITAMs (13 of 25 groups) develop a lethal, multiorgan autoimmune disease which is caused by a breakdown in negative selection. While there was a linear correlation between the number of wild type CD3 ITAMs and T cell proliferation, cytokine production was unaffected by ITAM number. Thus, a high ITAM number imparts on the TCR:CD3 complex the unique ability to provide scalable signaling that can modulate proliferation, and yet ensure effective negative selection and the prevention of autoimmunity. Expression of the T cell receptor (TCR):CD3 complex is tightly regulated during T cell development. Subtle changes in TCR signal strength determine the fate of immature thymocytes and thus fine control of TCR:CD3 expression level on immature thymocytes is likely to be essential in controlling T cell selection. The mechanism and physiological role of this regulation are unclear. We have shown that the TCR:CD3 complex is constitutively ubiquitylated in immature DP thymocytes, but not mature SP thymocytes or splenic T cells. Steady-state, tonic CD3 ubiquitylation is mediated by the RING-type E3 ubiquitin ligase c-Cbl, Src kinases, the CD3 ϵ PRS and occurs in the absence of MHC ligation. Blocking TCR:CD3 tonic ubiquitylation by mutating all the lysines in the CD3 cytoplasmic tails significantly up-regulates TCR levels on DP thymocytes, while mimicking mono-ubiquitylation by expression of a CD3 ζ -monoUb fusion molecule reduces TCR levels on immature thymocytes. Moreover, modulating CD3 ubiquitylation shifts the signaling threshold for positive and negative selection, and regulatory T cell development. Thus, tonic TCR:CD3 ubiquitylation results in precise regulation of TCR expression on immature T cells.

1. Holst J, Vignali KM, Burton AR, Vignali DAA (2006). Rapid analysis of T cell selection and function *in vivo* using T cell receptor retrogenic mice. ***Nature Methods* 3**:191-197.
2. Holst J, Wang H, Durick Eder K, Workman CJ, Boyd K, Baquet Z, Singh H, Forbes K, Chruscinski A, Smeyne R, van Oers NSC, Utz PJ, Vignali DAA (2008). T cell receptor:CD3 ITAM-mediated scalable signaling ensures effective negative selection and prevents autoimmunity. ***Nature Immunology* 9**:658-666.
3. Wang H, Matsuzawa A, Brown S, Zhou J-R, Guy CS, Tseng P-H, Forbes K, Nicholson TP, Sheppard PW, Haecker H, Karin M, Vignali DAA (2008). Analysis of non-degradative protein ubiquitylation with a monoclonal antibody specific for lysine 63-linked polyubiquitin. ***PNAS* 105**:20197-20202.