Natural killer T cell—a cat o’ nine lives!

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The cylindromatous gene *Cyld* encodes a deubiquitylase (DUB), which regulates NF- κ B activation. CYLD deficiency results in faulty innate and adaptive immune systems in mice, the basis for which remains incompletely understood. In this issue of *The EMBO Journal*, Sun and colleagues report that CYLD-deficient mice develop the innate-like lymphocyte called natural killer T (NKT) cells but lose them to death, which resulted from overactivation of NF- κ B and low interleukin-7 receptor (IL-7R) expression. Consequently, CYLD-deficient NKT cells poorly respond to IL-7 and do not upregulate inducible costimulatory (ICOS) receptor, molecules that serve as survival signals in other cellular contexts. These findings integrate disparate prior knowledge revealing the molecular basis for NKT cell homeostasis and have implications for understanding why human NKT cell frequency varies between individuals.

The tumour suppressor gene *Cyld* encodes a DUB that belongs to the ubiquitin-specific protease class. CYLD regulates a multitude of fundamental biologic processes including innate and adaptive immune responses. Its constitutive DUB activity regulates nuclear NF- κ B function by acting on multiple components within the pattern recognition receptor-NF- κ B and the T-cell receptor (TCR)-NF- κ B signalling axes whose activation depend on ubiquitylation. Uniquely, CYLD also acts on the lymphoid-specific kinase Lck to regulate the activation of its target Zap70. Thus, CYLD deficiency results in impaired thymocyte development as well as in faulty innate and adaptive immune functions (Sun, 2008). As NKT cells—a thymus-derived lymphocyte known to regulate antitumour and antimicrobial immunity as well as autoimmunity—require NF- κ B signalling for their ontogeny, homeostasis and function, Sun and colleagues now assessed the function of CYLD in these processes (Lee *et al.*, 2010).

Figure 1 CYLD regulates multiple survival signals within developing NKT cells. The function of CYLD (shown in red) in NKT cell ontogeny and maintenance (see text) is diagrammed against the backdrop of currently known factors (shown in grey) that regulate these processes within this lineage (Godfrey *et al.*, 2010). T-cell development begins with CD4CD8 double-negative (DN) thymocytes, which progress through four stages (not shown) to become CD4CD8 double-positive (DP) cells. NKT cell lineage commitment occurs with HEB (an E2A family transcription factor)-ROR γ t-dependent expression of the semi-invariant TCR at the DP stage. Hence, both NKT cells and conventional CD4 and CD8 T cells require the same factors to progress from the DN to DP thymocyte stage. Positive selection by DP thymocytes displaying CD1d-self-lipid complexes at stage 0/1 induces the expression of promyelocytic zinc finger protein (PLZF), and perhaps NF- κ B. Ontogenetic progression at this stage is further regulated by signalling lymphocyte activation molecule family member (SLAMF)-SLAM-associated protein (SAP)-Fyn (a Src kinase) signalling axes culminating in GATA3 and T-bet expression, which are transcription factors essential for the elaboration of IL-4 and interferon- γ for which this lineage is best known (stages 1 and 2). At these stages, TCR-induced calcineurin-NFAT-Egr2 and PKC θ -NF- κ B signalling axes have critical functions in lineage proliferation (e.g. Myc), maintenance (e.g. Bcl-x_L) and effector differentiation (e.g. Csrf). Finally, terminal maturation to stage 3 requires T-bet and IL-15 signalling. Dashed arrows, intermediates not known; thick arrow, over activation.

Although NKT cells have critical immunoregulatory functions *in vivo*, their frequency varies among different mouse strains as well as between individuals, especially among those that are predisposed to autoimmune diseases (Godfrey *et al*, 2010). Yet, the molecular basis for this variation remains an enigma. Over the years, a number of receptors, cytokines, signalling molecules and transcription factors were independently identified as regulators of NKT cell ontogeny, homeostasis and function (Figure 1; Godfrey *et al*, 2010). Although several essential pathways for NKT cell ontogeny and maintenance have been identified, how these diverse pathways are integrated in one well-orchestrated regulatory network remained unknown.

Lee *et al* found that CYLD-deficient mice develop NKT cells, which progress from progenitors (stage 0) of this lineage through the entire ontogenetic programme to give rise to mature NKT cells (stage 3; Figure 1). Yet, the overall frequency of these cells was very low in CYLD-deficient mice. On dissecting the basis of this deficiency, Lee *et al* found overt apoptosis at all developmental stages of this lineage. Moreover, CYLD deficiency resulted in unbridled classical NF- κ B activation within NKT cells, which was tempered by T cell-specific overexpression of a mutant incapable of signal-dependent phosphorylation of I κ B α (Lee *et al*, 2010). This is consistent with the earlier findings that (1) among other pathways, CYLD regulates the TCR-NF- κ B signalosome (Sun, 2008); (2) the TCR-PKC θ -NF- κ B axis regulates NKT cell homeostasis through the induction of the pro-survival factor Bcl-x_L (Stanic *et al*, 2004a,b) and (3) the overexpression of the constitutively active I κ B kinase-2 mutant results in the development of all stages of NKT cells yet maintains them only at low frequency (Cen *et al*, 2009). Together, these findings suggest that NF- κ B functions as a rheostat within developing NKT cells requiring an optimal threshold of NF- κ B activity for lineage survival.

Further mechanistic studies by Lee *et al* revealed that *Cyld*-null NKT cells express lower levels of IL-7R when compared with their wild-type counterpart. Hence, *Cyld*-null NKT cells poorly responded to IL-7 and phosphorylated STAT5 at tyrosine-694 and failed to induce ICOS (Lee *et al*, 2010). This phenotype is consistent with earlier studies, which

showed that IL-7R-deficient mice developed low numbers of mature NKT cells (Boesteanu *et al*, 1997). As IL-7R signalling is also critical for the first two stages (DN1–DN2) of thymocyte development, the function of IL-7R signalling in NKT cell ontogeny—which bifurcates at the DP stage (Figure 1; Godfrey *et al*, 2010)—went unnoticed. As IL-7R and Bcl2 are developmentally expressed within NKT cells (Godfrey *et al*, 2010), it is possible that IL-7 signalling induces Bcl2 to further regulate NKT cell survival.

ICOS was previously shown to regulate NKT cell development and homeostasis (Godfrey *et al*, 2010). As shown by Lee *et al*, IL-7 induced *Icos* expression in NKT cells that was interrupted in CYLD-deficient mice. Together, these data for the first time provide an integrated picture of how multiple signalling pathways cross-talk to aid the narrow escape of NKT cells from death (Lee *et al*, 2010). Perhaps such an integrated regulation is essential for the maintenance of a lineage that was designed to be self-reactive. So, the question becomes, what death signals are these cells escaping from? Clearly, it is not Fas–Fas ligand mediated (Lee *et al*, 2010). So, is it a signal emanating from the NKT cell receptor for antigen? If it is, is it because this TCR is constantly exposed to its ligand, CD1d displaying endogenous lipids, thereby over stimulating NF- κ B in the absence of CYLD's reign? If so, the function of CYLD in regulating the NF- κ B rheostat seems to be an important one for NKT cell development and homeostasis. Such a function raises the intriguing possibility that some of the known mutations/polymorphisms in human *CYLD* (Courtois, 2008) that temper its DUB activity and those within CYLD-regulated proteins (e.g. NEMO; Sun, 2008) could underlie varied (ranging from 0.01 to 3%) NKT cell frequencies within our population.

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Conflict of interest

The authors declare that they have no conflict of interest.

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