NATURAL KILLER CELLS

A new couple on the scene

Using an innovative method for downregulating natural killer (NK)-cell receptor NKG2D (NK group 2, member D), Medzhitov and colleagues have shown that the pathways triggered by this activating receptor are coupled to those that stem from the interleukin-15 receptor (IL-15R).

NK-cell activating receptors can interact with different signalling adaptors and, conversely, the adaptors can complex with multiple receptors, which in turn can be expressed by many subsets of NK cells. This promiscuity can thereby lead to a high degree of functional redundancy. Possibly for this reason, classical single-gene targeting strategies that ablate the signalling adaptors *Dap10* or *Dap12* have not indicated any significant roles for the products of these genes in NK-cell development and maintenance. This prompted the authors of this study to seek an alternative approach.

The authors created a transgenic mouse line in which DAP10, the signalling adaptor of NKG2D, was fused at its C-terminus to ubiquitin, thus flagging the protein for degradation. This method not only resulted in the ubiquitin-mediated degradation of DAP10 in NK cells from these mice, but also of its associated molecules.

Having therefore obtained a method whereby NKG2D expression could be substantially decreased, the authors set out to study the functional consequences of this reduction. The NKG2D-dependent cytotoxic response was defective, consistent with a downregulation of NKG2D, whereas signals from other activating and inhibitory receptors were not compromised. Importantly, transgenic mice had fewer NK cells overall than their wild-type counterparts.

As NK-cell numbers are also considerably lower in mice that lack components of the IL-15R signalling pathway compared with wild-type mice, the authors tested the IL-15 responsiveness of the DAP10-ubiquitin-expressing NK cells. They found that, contrary to wild-type cells, IL-15 did not promote NK-cell survival or propagation, and the transgenic NK cells had fewer transcripts of IL-15-induced genes. As the expression of IL-15R on the surface of the transgenic NK cells was not altered, it was speculated that the insensitivity to IL-15 may result from a defect in the transduction of the intracellular signals that stem from IL-15, and that DAP10 and its associated molecules may promote IL-15R signalling. Co-immunoprecipitation studies confirmed this by showing that DAP10 associates with both the γ- and β-chains of the IL-15R; however, the protein levels of JAK3 (Janus kinase 3) and STAT5 (signal transducer and activator of transcription 5), both of which function downstream of IL-15R, were normal and therefore the specific molecular basis for the IL-15R unresponsiveness still remains to be determined.

IL-15 has also been reported to promote NK-cell effector responses; so, could IL-15R be involved in the priming of DAP10 for the transduction of NKG2D-mediated signals? As the kinase responsible for phosphorylating the key tyrosine residue of DAP10 has not been identified, the authors tested whether JAK3 might exert this function. Indeed, JAK3 was found to be a kinase for DAP10 both *in vitro* and *in vivo*. More importantly, it was shown that activation of JAK3 mediated by IL-15 was necessary to prime DAP10 signalling, as indicated

by the increase in NKG2D-induced DAP10-dependent degranulation of NK cells in the presence of IL-15, and by the fact that the presence of a JAK3 inhibitor could abolish these effects.

This study shows that IL-15R signalling, by inducing the JAK3-dependent phosphorylation of DAP10, is required for the signal transduction and cytotoxicity that is mediated by NKG2D. The authors further suggest that the coupling of ubiquitously expressed signalling adaptors to other distinct cell-type-specific receptors could serve to deliver unique signals in different physiological contexts.

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ORIGINAL RESEARCH PAPER Horng, T., Bezbradica, J. S. & Medzhitov, R. NKG2D signaling is coupled to the interleukin 15 receptor signaling pathway. *Nature Immunol.* 21 October 2007 (doi:10.1038/ni1524)