NATURAL KILLER CELLS

A TACTILE restraint

The immunoglobulin superfamily member CD96 (also known as TACTILE) negatively controls cytokine production by natural killer (NK) cells, according to the first *in vivo* functional study of this receptor. Mark Smyth and colleagues show that this function of CD96 is relevant to the NK cell-induced pathology of lipopolysaccharide (LPS)-induced endotoxemia, as well as to NK cell-mediated regulation of tumour formation and metastasis.

CD96, together with CD226 and TIGIT, belongs to a family of receptors that interact with nectin and nectin-like ligands. CD226 and TIGIT have a common ligand, CD155 (also known as NECL5 and poliovirus receptor), and they reportedly counterbalance each other in terms of their regulation of NK cell function; CD226 activates NK cell-mediated cytotoxicity, whereas TIGIT is an inhibitory NK cell receptor. By contrast, little is known about the function of CD96, which is also expressed by NK cells and can also bind CD155.

The authors generated *Cd96*^{-/-} mice and showed that the numbers and phenotypes of the main immune cell subsets were normal. Following sub-maximal LPS challenge, *Cd96*^{-/-} mice had significantly decreased survival compared with wild-type mice, and this was associated with marked increases in the serum level

of interferon- γ (IFN γ) and in the proportion of IFN γ ⁺ splenic NK cells. Wild-type and *Cd96*^{-/-} mice survived equally well when they were depleted of NK cells before LPS injection, and *Cd96*^{-/-} NK cells that were transferred into immunodeficient recipient mice before LPS challenge produced more IFN γ compared with transferred wild-type NK cells, which shows that the function of CD96 in this model is NK cell intrinsic.

There was marked induction of CD155 expression on dendritic cells (DCs) and macrophages after LPS injection, and *Cd96*^{-/-} NK cells produced more IFN γ than wild-type NK cells when cultured with CD155⁺ bone marrow-derived DCs and LPS. These results indicate that CD96 inhibits IFN γ production by NK cells in response to CD155 induction by LPS. Whereas there was no effect of the absence of TIGIT on the response to LPS, *Cd226*^{-/-} mice had a decreased frequency of IFN γ ⁺ NK cells, which indicates that CD226 and CD96 have opposing roles in the regulation of NK cells in this model.

Although CD226 and CD96 can compete for binding to CD155 *in vitro*, the inhibitory function of CD96 *in vivo* was not a result of competitive depletion of CD155 from the activating receptor CD226, as shown by the ability of CD96 deficiency to increase the production of IFN γ

in *Cd226*^{-/-} mice. Confirming the direct effect of the CD155–CD96 interaction on NK cells, the production of IFN γ by wild-type NK cells in response to interleukin-12 (IL-12) and IL-18 was decreased in the presence of plate-bound CD155, and this effect could be reversed by a blocking antibody specific for CD96.

The counterbalancing effects of activating CD226 and inhibitory CD96 are also relevant to NK cell-mediated antitumour immunity. *Cd226*^{-/-} mice had more lung metastases than wild-type mice following injection of a melanoma cell line, whereas immunodeficient mice reconstituted with *Cd96*^{-/-} NK cells had fewer metastases than the mice reconstituted with wild-type NK cells. This host-protective effect of CD96 deficiency was dependent on IFN γ production by NK cells. Similarly, *Cd96*^{-/-} mice were more resistant than wild-type mice to chemically induced fibrosarcoma, in an IFN γ - and NK cell-dependent manner. Further studies of human NK cells will be required to determine the therapeutic potential of targeting CD96 to promote NK cell activity in the context of cancer.

Kirsty Minton

“
CD96
inhibits IFN γ
production
by NK cells
in response
to CD155
induction by
LPS”

ORIGINAL RESEARCH PAPER Chan, C. J. et al.
The receptors CD96 and CD226 oppose each
other in the regulation of natural killer cell
functions. *Nature Immunol.* <http://dx.doi.org/10.1038/ni.2850> (2014)