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NATURAL KILLER CELLS

Stop, look, listen

When a migratory natural killer (NK) cell encounters a potential target cell, it must stop and integrate signals from various sensory inputs to decide whether to proceed with a cytolytic response. Daniel Davis and colleagues show how the formation of a lytic synapse between an NK cell and a target cell, resulting in the polarized secretion of cytolytic granules, is continuously regulated by the balance between signalling through activating and inhibitory receptors.

Using an NK cell line transfected with the inhibitory receptor killer cell immunoglobulin-like receptor 2DL1 (KIR2DL1), they showed that the size of the contact area with target cells increased fourfold when these cells did not express any ligand for KIR2DL1 (an activating synapse) than when they expressed the inhibitory ligand HLA-Cw6 (an inhibitory synapse). At activating synapses in this and other systems, NK cells spread rapidly over the surface of the target cell and a ring of filamentous actin (F-actin) accumulated at the periphery of the activating synapse, which coincided with the polarization of cytolytic granules towards the target cell; at inhibitory synapses, NK cells spread less rapidly and an F-actin ring did not form.

NK cells mounted a symmetrical spreading response to glass slides coated with a monoclonal antibody specific for the activating receptor NKG2D. The percentage of cells that spread and formed an F-actin ring reduced with decreasing concentration of NKG2D-specific antibody, but the size of the area over which an individual cell spread was not dose dependent. The percentage of NK cells forming an F-actin ring in response to NKG2D-specific antibody could also be decreased in a dose-dependent manner by the addition of antibody specific for the inhibitory receptor NKG2A, but again the area of spreading in cells that did assemble an F-actin ring was not altered. This indicates that the decision to form an activating synapse and therefore to kill the target cell is an all or nothing event that is determined by the balance between activating and inhibitory signals, but is this decision irreversible?

Using slides coated with alternate stripes of NKG2D-specific antibodies and of NKG2D- and NKG2A-specific antibodies, they showed that NK cell spreading on regions of the slide with only the activating antibody was halted when the inhibitory antibody was encountered. For an individual NK cell, the intensity of F-actin staining was significantly higher in regions of the cell that were in contact with only activating antibody. The authors showed that NKG2D ligation delivers a 'stop' signal to migrating NK cells that prolongs the duration of conjugation; this can be reversed by the ligation of inhibitory receptors to allow the NK cell to move on to another potential target. These results indicate that signal integration occurs continuously during NK cell spreading and there is no irreversible commitment to form an activating synapse, which could be important to prevent cytolytic responses from being misdirected to healthy cells that closely neighbour the target cells.

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ORIGINAL RESEARCH PAPER Culley, F. J. *et al.* Natural killer cell signal integration balances synapse symmetry and migration. *PLoS Biol.* **7**, e1000159 (2009)