

SPOTLIGHT

Natural killers shed attachments to kill again

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Natural killer cells target antibody-bound cells following engagement of the Fc receptor CD16. Srpan et al. (2018. *J. Cell Biol.* <https://doi.org/10.1083/jcb.201712085>) reveal that activation-induced shedding of CD16 leads to more motile behavior, allowing more targets to be engaged and killed in a given time.

Alongside T and B cells, natural killer (NK) cells belong to the lymphocyte subset of white blood cells; however, they are distinct from the other lymphocytes in that they form part of the innate immune system. They use combinations of germline-encoded activatory and inhibitory receptors to target virally infected and dysregulated cells, leading to innate, or “natural,” killing, and also serve as effector arms of adaptive immunity by targeting antibody-coated cells through the Fc receptor CD16 (also known as FcγRIII). Cells identified as targets are killed by the focused secretion onto their surface of preformed granules of cytolytic effector molecules, including perforin, which permeabilizes the target membrane, and granzymes, which have a range of proapoptotic and cytotoxic effects.

The highly cytotoxic nature of NK cell lytic granules requires that their release be tightly regulated toward the appropriate cell, and hence degranulation occurs only upon direct contact with a target. Of the germline-encoded receptors that mediate this, some recognize common pathogen-derived antigen proteins (e.g., the natural cytotoxicity receptors), whereas others bind human proteins that are markers for cellular stress (e.g., MICA and MICB, commonly expressed on cancerous cells and recognized by the C-type lectin NKG2D) or markers of targeting by the adaptive immune system (i.e., CD16). Engagement of these activatory receptors leads to the formation of an organized interaction between the NK cell and its target, known as a cytolytic immunological synapse. This integrates signals into the NK cell and acts as a site of lytic granule delivery. Immunological synapses were first described between T cells and antigen-presenting cells; however, various immunological synapses have since been observed between other immune cells and between immune and nonimmune cells. Characteristic features of these structures include extensive remodeling of the cytoskeleton and accumulation of filamentous actin, recruitment of signaling and adhesion molecules and intercellular transfer of secreted proteins and/or vesicles. The most similar synapse to the NK cell synapse is that of cytotoxic T cells, which use similar cytolytic granules to kill infected or cancerous cells.

In this issue, Srpan et al. addressed the question of how NK cell stimulation through the different types of activatory receptors influence release of cytotoxic agents such as perforin and killing of subsequent target cells. By capturing and quantifying released perforin on a single-cell level, they observed that repeated stimulation of human primary NK cells through activation of either CD16 or NKG2D led to a progressive reduction in perforin release. Intracellular perforin stores were also reduced after each degranulation event, which one might assume indicates simple cell exhaustion following multiple degranulations. Interestingly, however, robust perforin release was recovered in cells stimulated through CD16 if they were subsequently activated through NKG2D. Conversely, activating cells in the inverse order (i.e., NKG2D then CD16) did not restore high levels of perforin secretion. This was caused by reduced surface CD16 in response to activation through either receptor, whereas NKG2D expression was down-regulated only by its own activation, not CD16 ligation. This indicates for the first time that the order of NK cell contacts influences subsequent responses. It also suggests a hierarchy of receptors, where NKG2D can regulate CD16 but not vice versa. Signaling from both CD16 and NKG2D increases the surface activity of the metalloprotease ADAM17, which specifically cleaves CD16 to shed its antibody-binding ectodomain (Romee et al., 2013). In contrast, down-regulation of NKG2D occurs through ubiquitin-mediated endocytic recycling (Molfetta et al., 2016) that does not appear to be activated by CD16 signaling.

Nonetheless, as little as 1% of the granule contents of an NK cell is sufficient to kill a target (Gwalani and Orange, 2018), so the critical threshold for regulation may be quite high. Through coinubation of human primary NK cells with antibody-coated or MICA-expressing cells, Srpan et al. (2018) observed that target killing was strongly affected by the order of contact. Killing of antibody-coated cells reduced by a third when NK cells had previously encountered MICA-expressing cells, whereas exposure to antibody-coated cells did not affect subsequent killing of those expressing MICA. To track the encounters of individual NK

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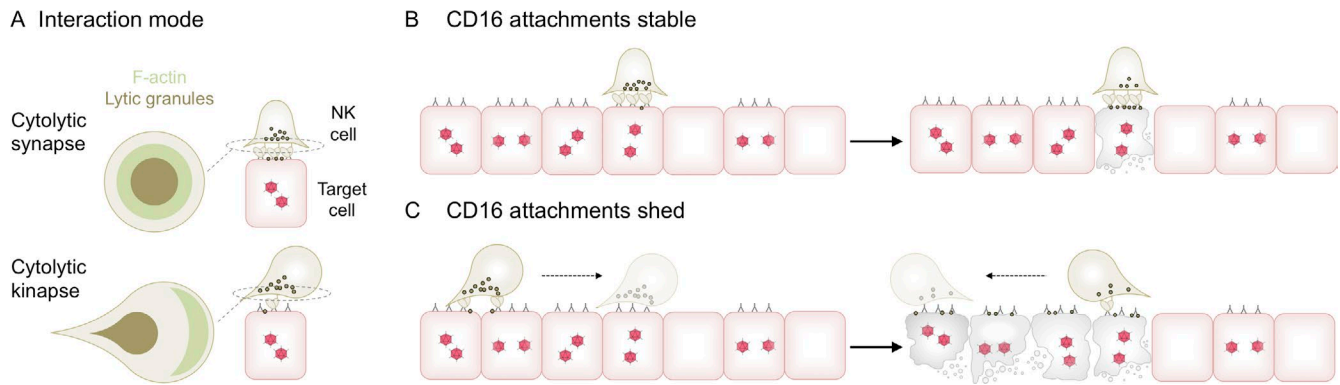


Figure 1. NK cell killing can be achieved through stable or mobile contacts. (A) Synapses are symmetrical, stable structures formed between NK cells and target cells, whereas kinapses are polarized, motile interactions. (B) Persistent interaction of NK-bound CD16 with target-bound antibody promotes a synaptic structure. (C) Conversely, activation-induced shedding of CD16 allows NK cells to adopt more kinaptic structures, shortening contacts with individual cells while promoting durable contact with a continuous target tissue and increasing the overall rate of killing. It is not clear whether kinapses form on first engagement of CD16 or emerge from synapses formed with originally encountered cells.

cells, Srpan et al. (2018) observed cells in microwells using time-lapse microscopy. NK cells first engaging a MICA-expressing cell became extremely refractory to killing of a subsequent antibody-coated cell. Indeed, activation of NKG2D resulted in even more potent CD16 inhibition than activation of CD16 itself, since initial killing of an antibody-coated cell did not reduce killing of either a second such cell or one expressing MICA.

Why, then, would NK cells make themselves less conducive to antibody-driven killing during successive engagements, particularly when serial killing by individual cells is important for robust population-level killing (Choi and Mitchison, 2013; Vanherberghen et al., 2013)? Typically the loss of CD16 has been interpreted as detrimental to NK cell responses. Multiple studies have suggested that large CD16^{low} NK cell populations persist in chronic infections such as HIV (e.g., Liu et al., 2009), and therapeutic inhibition of CD16 cleavage serves to increase NK cell activity in such scenarios and during cancer immunotherapy (Zhou et al., 2013). However, Srpan et al. (2018) observed that inhibition of ADAM17 substantially decreased the rate of NK cell detachment from target cells, without affecting the killing efficiency of an individual contact. A similar effect was observed in NK cells expressing an uncleavable CD16 mutant, reducing the proportion of cells releasing killed targets from 70% to 10%. Consequently, each NK cell was capable of killing fewer target cells in a given timeframe, instead becoming stalled in long-lived contacts (several hours) with a small number of cells. Srpan et al. (2018) observed that, within a 3D matrix, NK cells frequently formed contacts with three or more cells in an 8-h period, but this was reduced substantially by inhibition of ADAM17, whereas death of NK cells in contact with targets increased from 23% to 38%.

CD16 cleavage did not simply increase detachment after killing, but also influenced the underlying structure of the cell-cell contact. In examining the organization of filamentous actin, which accumulates at the periphery of stable immunological synapses (Fig. 1 A), Srpan et al. (2018) noted that NK cells on antibody-coated surfaces typically formed asymmetric contacts, termed “kinapses,” and migrated rapidly, whereas those on MICA-coated surfaces formed synapses and were relatively

static. Inhibition of CD16 shedding promoted more stable, static synapses. Kinapses are motile contacts that can lead to durable (several hours) or transient interactions depending on whether the recognition system driving adhesion and polarization is continuous or spatially distinct (Mayya et al., 2018). Thus, the spatial distribution of target cells in tissues *in vivo* may also impact whether NKG2D or CD16 engagement leads to serial encounters or more durable interactions. NK cells targeting tumor cells though NKG2D *in vivo* form kinapses rather than synapses (Deguine et al., 2010), in contrast to the *in vitro* observations of Srpan et al. (2018). Interestingly, simultaneous engagement of CD16 and NKG2D in the same system induced more synapse-like interactions (Deguine et al., 2012), though changes in CD16 or NKG2D expression in the tumor-infiltrating NK cells was not investigated.

Srpan et al. (2018) therefore provide a new conceptual framework in which to view the design and interpretation of experiments to understand and exploit the interplay between killing efficiency and kinapse-dependent tumor penetration in immunotherapy. Their observations also raise a number of further questions. Why is CD16 seemingly the only NK cell receptor to be regulated in this manner? Shedding of NKG2D ligands MICA and MICB (which are also cleaved by ADAM17; Chitadze et al., 2013) may have a comparable effect *in vivo*, or perhaps CD16 uniquely requires receptor-specific shedding because the strength of the antibody-antigen interaction is variable and cannot be controlled for. Do CD16-mediated contacts inherently result in kinapses or is the first encounter more synaptic in nature? Are CD16^{low} populations in infection or cancer indications of impaired or highly active NK cell responses? Further study of the nature of NK cell kinapse dynamics, particularly *in vivo*, will be required to answer these and other outstanding questions.

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