

Killing of tumor cells by NK cells with

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What are NK cells and how do they kill tumor cells?

Hans-Gustaf Ljunggren: Natural killer (NK) cells are innate immune cells that were first characterized for their capacity to kill tumor cells without the need for prior immunization. This initial observation gave them their name (1). Most mature NK cells circulate in blood in a partially activated “ready-to-go”-state. They are fully activated by cytokines and/or by interaction with specific molecules expressed on target cells, e.g. tumor cells. This leads to induction of effector functions. Major effector functions

of NK cells are direct cytotoxicity and cytokine production (2). Exocytosis of lytic granules containing the membrane-disrupting protein perforin and a family of structurally related serin proteases (known as granzymes) is considered the major mechanism used by NK cells to kill target cells. Both contribute to apoptosis of the target cell. NK cells also express molecules of the tumor necrosis factor (TNF) superfamily that can engage death receptors on target cells, resulting in apoptosis (3).

Why do NK cells need a large number of receptors?

Hans-Gustaf Ljunggren: NK cells express a large number of cell surface receptors that recognize different ligands on target cells. From an evolutionary perspective, receptor expression has likely been driven by pathogen encounter and perhaps also by other mechanisms. The rather recent identification of these receptors and their ligands has in large uncovered the molecular mechanisms that regulate NK cell activation and function (3,4). Several activating and co-stimulatory receptors have been identified that, once engaged, induce cytotoxicity and cytokine production. Activation of NK cells is under continuous control by inhibitory receptors that, upon specific ligand interaction, prevent reactions against healthy cells or tissues. A common denominator for many activating and co-stimulatory receptors is that they bind self-molecules induced upon cellular stress. Co-stimulatory receptors modify and/or strengthen the responses by activation receptors but do not activate NK cells by themselves. The rather large

panel of activation receptors that exist provide a possibility to react against different ligands, and a backup for potential immune escape. A common denominator for inhibitory receptors is that they bind classical and non-classical major histocompatibility complex (MHC) class I molecules. These molecules are normally expressed on most healthy cells in the body, but expression is often lost upon transformation or other forms of cellular stress. Many receptors, such as the killer cell immunoglobulin-like receptors (KIRs), are expressed on subsets of NK cells, and many NK cells express only few of potentially many different types of receptors. Variation in KIR receptor expression between different individuals is significant. One hallmark of the KIR inhibitory receptor system is that it creates a system allowing NK cells to detect cells lacking expression of single MHC class I alleles. A situation observed in some cancers, and that could be potentially beneficial in settings of stem cell transplantation (STC) against cancer.

What is the role of cytokines?

Hans-Gustaf Ljunggren: More knowledge is needed on the role of cytokines in promoting or suppressing tumor immunity (5). *In vitro* and *in vivo*, NK cells can be stimulated by cytokines such as IL-2, IL-12, IL-15, IL-18, IL-21 and type 1 interferons (IFN α/β). Once activated, NK cells show increased cytolytic, secretory and proliferative functions. Cytokines released by

activated NK cells include IFN- γ , GM-CSF and TNF- α . Together with secreted chemokines, these cytokines stimulate inflammatory responses. The cytokines can modulate monocyte, dendritic cell, and granulocyte growth and differentiation. They can also influence acquired immune responses. IFN- γ may also affect host responses to tumors by restricting angiogenesis (2-5).

How can NK cells be used to kill tumor cells in a clinical setting?

Hans-Gustaf Ljunggren: Two principle strategies for therapeutic use of NK cells exist. The first involves activation of endogenous NK cells, and the second is based on adoptive transfer of NK cells (6,7). In addition to this, graft-vs-tumor effects mediated by developing NK cells in the donor following hematopoietic SCT may be considered as a third strategy (8). Various clinical protocols for endogenous NK cell activation have been initiated based on cytokine-therapy. Other forms of *in vivo* modulation of NK cell function have more recently also raised interest (7). Early clinical trials based on adoptive transfer of autologous IL-2 activated NK cells have been met with, at most, very limited clinical effects (6). Recently, however, a partially successful trial has been based on a preconditioning regimen of patients with acute myeloid leukemia (AML) in combination with infused haploidentical IL-2 activated NK cells (8). This study was inspired by earlier results in settings of haploidentical hematopoietic SCT to patients with AML (8,10). The rationale in both these settings is that in certain donor-recipient conditions, subsets of donor NK cells will lack inhibitory receptors for host MHC class I molecules, providing a condition for a potential graft-vs-leukemia effect (i.e., “missing-self” reactivity, ref 11). Different

ways to modulate the expression of NK cell receptors on fresh NK cells or on NK cell lines (silencing and/or overexpression of specific receptor genes as well as modulation of cell surface expression by antibodies or other means) is clearly of interest in future attempts to enhance NK cell anti-tumor responses. Finally, it should be stated that NK cell therapy against solid tumors represent special problems, including not seldom the size of the tumor *per se*, and the presumed necessity of NK cells to migrate to the tumor tissue and to infiltrate the tumor (6). Forces that impede NK cell function may likely operate in many situations. Likewise, immune escape mechanisms operate for NK cells as well as they do for T cells (12). Nonetheless, NK cell may likely find a role in future therapies of some tumors, either alone or in combination with other therapies.

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