



tion of the rate of appearance of one or another specific cargo proteins in CSF after $^2\text{H}_2\text{O}$ labeling could reveal neuron-specific defects that occur early in a disease process. More generally, stable isotope labeling of precursor pools followed by either cross-sectional or longitudinal CSF sampling offers a powerful tool to explore aspects of pathologic processes in the brain that are unlikely to be detected by either proteomic or metabolomic measures of absolute steady-state concentrations. Combining measures of total analyte and rates of appearance of labeled newly synthesized forms should increase our ability to define early stages of neurodegenerative diseases and facilitate the development and testing of novel therapies targeted to the pathologic process. In other words, treating the biomarker (e.g., cholesterol) may offer a path to prevent-

ing or delaying the clinical symptoms that lie downstream of that biomarker and are often irreversible consequences of failing to intervene early.

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When NK cells overcome their lack of education

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Cells of the immune system have evolved various molecular mechanisms to sense their environment and react to alterations of self. NK cells are lymphocytes with effector and regulatory functions, which are remarkably adaptable to changes in self. In a study published in this issue of the JCI, Tarek and colleagues report the clinical benefits of manipulating NK cell adaptation to self in an innovative mAb-based therapy against neuroblastoma (NB). This novel therapeutic strategy should stimulate further research on NK cell therapies.

NK cells are involved in the elimination of tumor cells and infected cells (1); they can kill their cellular targets via cytotoxic granule exocytosis and also secrete cytokines such as IFN- γ that participate in the shaping of the adaptive immune response. NK cells express a wide range of surface molecules that include inhibitory and activating receptors; in humans, this family comprises the natural cytotoxicity receptors (NKP30, NKP44, and NKP46), the Fc γ receptor IIIA (CD16), and the activating killer cell immunoglobulin-like receptors (KIRs). CD16 endows NK cells with antibody-dependent cell-mediated cytotoxicity

(ADCC) properties. These activating receptors associate with immunoreceptor tyrosine-based activation motif-bearing adaptors to transduce potent activating signals. The inhibitory KIRs in humans (and their functional homologs in mice, the Ly49 receptors) recognize classical major histocompatibility complex class Ia molecules (MHC-I) and transduce inhibitory signals via their intracytoplasmic tyrosine-based inhibition motifs. Upon NK cell encounter with potential target cells, the integration of activating and inhibitory signals dictates the NK cell response. Normal self cells expressing high levels of MHC-I and low levels of activating ligands are spared, while stressed cells with downregulated MHC-I expression and high levels of activating ligands activate NK cells and are killed.

NK cell education

The functional maturation of NK cells includes a process of education, also referred as to licensing, arming, or tuning, by which NK cells acquire effector functions that are adapted to the host in which they develop (2–5). In addition to its well-known role in the regulation of NK cell effector functions, MHC-I recognition by inhibitory receptors is also involved in NK cell education. When NK cells cannot sense self MHC-I, as in MHC-I-deficient mice or patients, individuals do not develop autoimmune disorders, and the NK cells are hyporesponsive to stimulation in vitro (Figure 1A) (6–8). Various conflicting models have been proposed to describe how the interaction of MHC-I-specific receptors with their ligands contributes to NK cell education (2–5). A unifying model inspired from the arming/disarming model initially proposed by David Raulet and colleagues (2) is consistent with the experimental data published so far. In this scenario, NK cells sense target cells via the combined engagement of activating, inhibitory, and adhesion receptors. The intensity of the NK cell response is commensurate with the inte-

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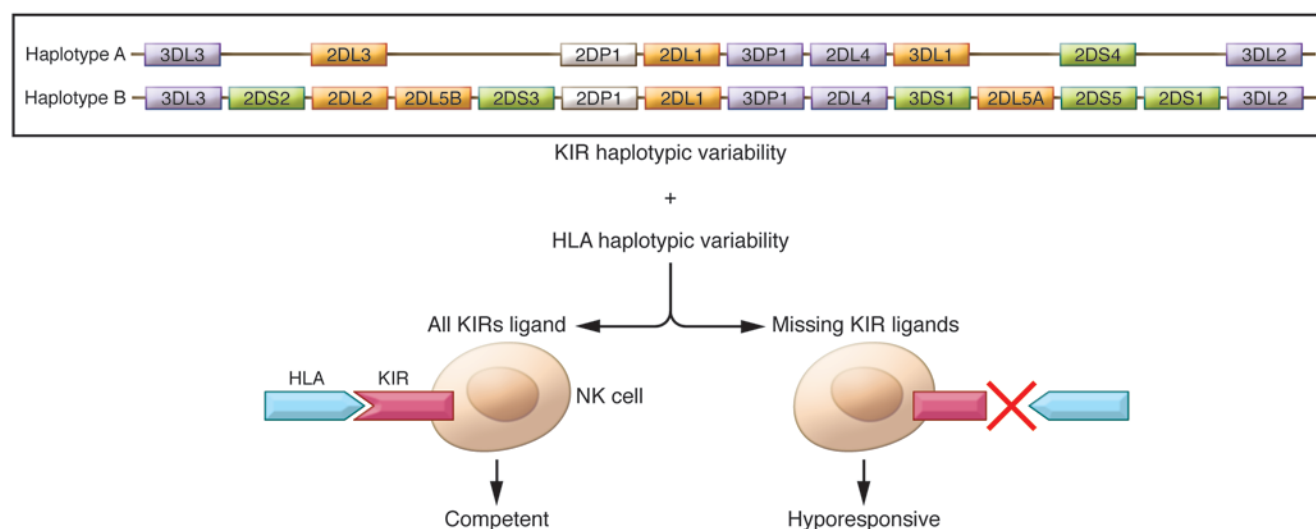


Activation of educated NK cells can be blocked through their inhibitory KIR receptors. **(A)** NK cell tolerance toward self cells is ensured by two mechanisms: MHC-I recognition by inhibitory KIR receptors that prevents activation of educated NK cells (top) and by the hyporeactivity of uneducated NK cells (bottom). **(B)** In the context of anti-GD2 (3F8) treatment, NB cells expressing GD2 will be decorated with this mAb. The activating receptor CD16, expressed on NK cells, recognizes 3F8, but only uneducated NK cells will mediate strong ADCC (bottom), as the engagement of inhibitory KIR receptors by their cognate HLA ligands expressed on NB blocks the activation of educated NK cells (top).

actin meshwork structures in uneducated NK cells (12). The fact that MHC-I-dependent education does not trigger profound and permanent modifications in the developmental program of NK cells (12) renders this process more versatile than initially thought. Adoptive transfer experiments have shown that NK cells expressing inhibitory receptors can switch from a hyporesponsive to a competent status upon recognition of the cognate MHC-I molecule, highlighting the constant adjustment of the NK cell reactivity to its surrounding environment (13, 14).

In this issue of the *JCI*, Tarek and colleagues have analyzed the contribution of educated and uneducated NK cells (that they refer as to licensed and unlicensed NK cells, respectively) in the killing of neuroblastoma (NB) tumors upon treatment with 3F8, a mAb targeting the disialoganglioside surface antigen GD2 (15). They genotyped patients with NB and divided the cohort into two groups: those lacking any HLA ligand for inhibitory KIRs (referred to as “missing KIR ligand” patients) and those with all HLA ligands for inhibitory KIRs (referred to as “all ligands present” patients). Fourteen *KIR* genes clustered in chromosome 19q13.4 have been described in humans.

four of which encode inhibitory cell surface receptors that interact with a specific group of MHC-I molecules (Figure 2 and ref. 16). This locus is subjected to extensive genetic variation, both in the number of genes present and in the sequence of each *KIR* allele (16). An additional level of complexity arises from the variegated expression of *KIR* genes in NK cell clones, leading to a vast diversity of NK cells present within each individual. As a consequence of these mechanisms and the absence of cosegregation of *KIR* and *HLA* genes, a fraction of NK cells may lack inhibitory receptors specific for self-MHC-I in each individual. Given that the interaction between inhibitory KIRs and their HLA ligands educates NK cells to acquire their full reactivity, the fraction of NK cells in the missing KIR ligand patients that only expresses inhibitory KIRs that have no cognate HLA ligand is hyporeactive in vitro (Figure 2). In contrast, all NK cells are educated in all ligands present patients. Therefore, the overall NK cell reactivity in these patients should be greater than that in missing KIR ligand patients. However, Tarek et al. found that patients with missing KIR ligands showed improved overall and progression-free survival when treated with 3F8. In vitro, both educated and uneducated NK cells were activated against NB targets by the 3F8 treatment through CD16, but educated

**Figure 2**

Some individuals express inhibitory KIR but not their HLA ligands. Schematic view of haplotypes A and B of the KIR locus representing genes encoding activating receptors (green), inhibitory receptors (orange), pseudogenes (white), and conserved genes (purple) that are pseudogenes or encode activating or inhibitory receptors. As the genes encoding the cognate HLA ligands are present on another chromosome and segregate independently of the KIRs, not all individuals express the cognate HLA ligands for their KIRs. In individuals expressing the KIRs and their HLA ligands (all KIR ligands), all NK cells are educated by the KIR/HLA interaction and are thus competent (bottom left). By contrast, in individuals that do not express the cognate HLA ligand (missing KIR ligands), NK cells cannot be educated and are hyporesponsive (bottom right).

NK cells were selectively inhibited by HLA ligands expressed on NK targets. Tarek and colleagues thus demonstrate that KIR-mediated inhibition upon interaction with its HLA ligand on target cells overcomes the responsive advantage delivered during the education process (15). These observations are consistent with a previous study in the mouse showing that uneducated NK cells are critical for the response against mouse cytomegalovirus (17). Taken together, these studies reveal that during the course of cancer or viral infection, uneducated NK cells can have a dominant and beneficial impact, challenging the association of the absence of education and hyporeactivity. Thus, the calibration of NK cell reactivity resulting from MHC-I-dependent education is sufficient to prevent NK cell autoreactivity at steady state, but it can be overridden in stress conditions (tumors, microbial infections) and upon treatment with therapeutic antibodies.

Could educated NK cells be rendered as efficient as uneducated NK cells?

Several strategies can be envisioned to boost NK cell reactivity against tumors in patients. The use of therapeutic therapies combining a tumor-targeting antibody with a second antibody specifically activat-

ing NK cells should be of particular interest. One way to stimulate effector NK cells is the use of anti-CD137 (4-1BB) mAb, which has shown promising ADCC boost when used in combination with rituximab (anti-CD20) or trastuzumab (anti-HER2) (18, 19). In NB, the persistent expression of the self-MHC-I on tumor cells is a key factor impairing the full activation of NK cells expressing self-MHC-I-specific inhibitory receptors. Thus, another promising approach to prevent the MHC-I-mediated inhibition of educated NK cells is to block inhibitory KIRs with a mAb (20). Coupling the anti-GD2 and anti-KIR mAb treatment is an attractive possibility to activate the entire NK cell population and to improve the overall survival of patients with NB and notably of those in which all KIR ligands are present.

Perspectives

Observations by Tarek and colleagues can be seen from two different perspectives. From the classical target cell recognition point of view, their findings are consistent with the inhibitory role played by KIRs, i.e., NK cells are more potent when relieved of MHC-I-dependent inhibition. However, from the education point of view, NK cells expressing KIRs which recognize MHC-I

ligand are educated and are expected to display greater reactivity than uneducated NK cells. Tarek and colleagues demonstrated that in given stimulatory conditions the KIR-mediated inhibition is a strong enough signal to prevail over the responsive advantage delivered during the education process. That uneducated NK cells are not permanently hyporesponsive and can dominate antitumor responses supports the development of strategies targeting educated NK cells, such as the anti-KIR mAb, and suggests that impacting on the education process of NK cells might not be necessarily prohibitive.

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NPARM in PHOX2B: why some things just should not be expanded

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Although the neural crest and its derivatives have been studied for a very long time, disorders of derivatives of the crest, the neurocristopathies, are not well understood. In this issue of the JCI, Nagashimada et al. provide an elegant analysis of one neurocristopathy, the association of neuroblastoma (NB) with Hirschsprung disease (HSCR) (aganglionosis of the terminal bowel) and congenital central hypoventilation syndrome (CCHS) (also known as NB-HSCR-CCHS), linked to mutations in PHOX2B. In a mouse model, Nagashimada et al. demonstrate that a disease-linked mutation promotes tumorigenesis and disrupts neurogenesis, sympathetic gangliogenesis, and crest cell colonization of the terminal bowel. They also show that mutant PHOX2B results in decreased proliferation of crest-derived cells and the development of glia at the expense of neurons. The work raises intriguing issues about the possible common origin of sympathetic and enteric nervous systems and provides new hope that we may someday understand the vexing abnormalities in gastrointestinal function that persist after the surgical treatment of HSCR.

The neural crest has long been recognized to be a gift that evolution has given to developmental biologists. It is a transient structure comprising cells that are probably heterogeneous (1), but that, as a unit, is multipotent, giving rise to melanocytes, Schwann cells, sympathetic neurons, parasympathetic neurons, enteric neurons,

enteric glia, endocrine cells, fibroblasts, muscle, bone, cartilage, and meninges (2). The neural crest thus provides opportunities to study the cellular and molecular mechanisms of epithelial-mesenchymal transformation (and the reverse), migration, aggregation, and differentiation. It has been a gift that just keeps on giving. Despite the developmental biological treasure that the neural crest has provided, however, the disorders that stem from neural crest dysfunction, the neurocristopathies (3), remain vexatious.

In this issue, Nagashimada et al. (4), in a truly remarkable study, have now provided a real insight into how a mutation in *PHOX2B*, a gene that encodes a paired homeodomain transcription factor that plays a critical role in the development of crest-derived autonomic neurons (5), can act in different cells in a gain-of-function or in a loss-of function manner. The gain-of-function activity is tumorigenic, causing neuroblastomas (NBs) to arise in the sympathetic nervous system, while perversely synergizing with the loss-of-function effect to disrupt neurogenesis, sympathetic gangliogenesis, and crest cell colonization of the terminal bowel, which becomes aganglionic (Hirschsprung disease [HSCR]). The faults in formation of neurons and ganglia appear to be the result of a failure of the reciprocal inactivation of *PHOX2B* and *SOX10*.

During normal development, crest-derived precursors of sympathetic and enteric neurons initially express *SOX10*, but acquire *PHOX2B* when they enter the pre-aortic (6) or enteric mesenchyme (7–9). Bipotent progenitors express both *PHOX2B* and *SOX10*, but *SOX10* is inactivated in cells that are destined to form neurons, and *PHOX2B* is inactivated in

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