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## **Inhibitory Receptors and Their Mode of Action: Key Insights from NK Cells**

Werner Held

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## Inhibitory Receptors and Their Mode of Action: Key Insights from NK Cells

Werner Held

During the time when attention was focused on how immune cells could be stimulated and costimulated, a small group of immunologists was preoccupied with the question of whether inhibition was a key feature of the regulation of NK cell function. In this context, the paper by Burshtyn et al. (1) provided crucial new information regarding receptor-mediated inhibition and how such receptors transduce signals that counteract cellular activation.

Two articles previously highlighted in the *Pillars* series had set the stage for the findings reported by Burshtyn et al. Target cells lacking MHC class I molecules were found to be susceptible to NK cell–mediated cytotoxicity, whereas cells expressing MHC class I were resistant (2), observations that led to the formulation of the missing self hypothesis. One interpretation of these findings was that MHC class I expressed on target cells inhibits NK cells (receptor inhibition). If so, the absence of target cell MHC class I expression relieves NK cells from inhibition, thus allowing NK cells to become activated. An alternative explanation was that MHC class I expression masks target cell Ags that are recognized by activating NK cell receptors (target interference).

The receptor inhibition model became widely accepted following the molecular identification of an NK cell receptor that prevented the lysis of target cells expressing certain MHC class I alleles (3). Puzzling at that time, the relevant mouse receptors were C-type lectin-like Ly49 family members, whereas human receptors endowed with the corresponding function were Ig-like (killer Ig-like receptors, or KIRs) (4–7). Gene transfer experiments and transgenic mice demonstrated that single receptors of either type conferred both the specificity for MHC class I molecules and the capacity to prevent target cell lysis (8, 9). According to the receptor inhibition model, lysis occurs when inhibitory receptors fail to interact with MHC class I on target cells. This implied the existence of other receptor/ligand interactions that trigger NK cell–mediated target cell lysis. The idea was that activating signals are counteracted locally by negative signals derived from inhibitory receptors. However, it was not known what “makes” these receptors

inhibitory and how they prevent the activation of the cytolytic response, two key points addressed by the Long laboratory.

Sequence comparisons of the cytoplasmic tails of the newly cloned NK cell receptors with inhibitory function provided the first insight into this issue. It turned out that inhibitory KIRs contain motifs in their cytoplasmic domains that resemble ITAM sequences present in the signaling adaptors of activating Ag receptors (YxxLx<sub>6–8</sub>YxxL, in single-letter amino acid code, with x indicating nonconserved positions). However, the spacing between the relevant tyrosine residues of KIRs is significantly greater and each subunit of the homodimeric Ly49 receptors contains only a single tyrosine. Despite these differences, both ITAM and the inhibitory receptor tails contain YxxL motifs, which raised the possibility that the cytoplasmic part of inhibitory receptors associates with proteins containing Src homology 2 (SH2) domains. Because cellular activation by Ag receptor complexes is mediated by tyrosine phosphorylation, SH2 domain–containing tyrosine phosphatases seemed attractive candidates for attenuating cellular activation.

The above hypothesis turned out to be correct and it was found that SH2 domain–containing phosphatase (SHP)-1 (HCP, PTP1C) associates directly with human KIRs, that the interaction is dependent on KIR phosphorylation, and that phosphopeptides corresponding to either one of the two tyrosine residues in the KIR cytoplasmic tail activate SHP-1 in vitro (1). Additional groups soon reported similar findings, extended them to Ly49 receptors, and identified SHP-2 (PTP1D) as an additional phosphatase associated with the cytoplasmic tail of inhibitory receptors (10–12). The key experiment to demonstrate the relevance of SHP-1 was the expression of a catalytically inactive form of SHP-1 in NK cell clones. This prevented the transduction of inhibitory signals by KIR and consequently resulted in target cell lysis despite the fact that the inhibitory receptor engaged MHC class I (1). The dominant interfering effect of the mutant SHP-1 suggested that the phosphatase activity of SHP-1 is required for KIR function. A role for SHP-1 was subsequently confirmed in vivo using NK cells from viable motheaten mice, a mouse strain with reduced SHP-1 activity (13).

Finally, the study from the Long laboratory, together with prior work from the Cambier (14) and Fearon (15) laboratories on the negative regulation of BCR signaling by FcγRIIb and CD22, firmly established a conserved inhibitory signature sequence (I/VxYxxL/V), which was termed immunoreceptor tyrosine-based inhibitory motif (ITIM). This sequence subsequently enabled the identification of numerous additional receptors and receptor families that were correctly predicted to mediate inhibitory function.

Department of Oncology, Ludwig Center for Cancer Research, University of Lausanne, 1066 Epalinges, Switzerland

Address correspondence and reprint requests to Dr. Werner Held, Department of Oncology, Ludwig Center for Cancer Research, University of Lausanne, Chemin des Boveresses 155, 1066 Epalinges, Switzerland. E-mail address: Werner.Held@unil.ch

Abbreviations used in this article: KIR, killer Ig-like receptor; SH2, Src homology 2; SHP, Src homology 2 domain–containing phosphatase.

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Today the integration of activating and inhibitory signals in NK cells is understood in much more detail (for a recent review, see Ref. 16), and it is clear that the attenuation of inductive signals plays important roles in the regulation of most immune cells. In addition to the canonical ITIM/SHP pathway, further inhibitory mechanisms have been discovered. The cytoplasmic domain of many inhibitory receptors contains immunoreceptor tyrosine-based switch motifs (ITSMs) (S/TxYxxV/I) (17), which can mediate inhibitory or activating signaling depending on specific cellular contexts. Moreover, there is evidence for inhibitory signaling pathways that operate independently of ITIMs and ITSMs. Finally, inhibition can also ensue based on competition between inhibitory receptors (e.g., CTLA-4) and activating receptors (e.g., CD28) for ligand binding (in this case, B7). Irrespective of the precise mechanism, the key function of inhibitory and coinhibitory receptors is to prevent autoaggression. Thus, strategies to enhance the activity of inhibitory receptor activity may be useful to dampen the activation of autoreactive immune cells. Conversely, blockade of inhibitory receptor pathways can be exploited to improve immune responses. KIR blockade is being tested for its efficacy to improve NK cell responses to malignant cells in leukemia patients (18). Abs blocking inhibitory receptors such as CTLA-4 and PD-1 are used to restore the function of exhausted T cells and have shown promising effects in cancer patients (19). Cellular inhibition and disinhibition have definitely reached the spotlight.

## Disclosures

The author has no financial conflicts of interest.

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