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The First Molecular Basis of the "Missing Self" Hypothesis

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I still remember the day my advisor, Dr. Rafael Solana, came to me with the article of Karlhofer et al. (1) in his hands and told me that that paper was the proof for the receptor inhibition model of the "missing self" hypothesis. I had just joined the Laboratory of Immunology in the University of Córdoba, in Spain, and my knowledge about NK cells was very basic. At that moment, I did not realize the whole significance of Yokoyama's work, but as time went by, I fully understood the breakthrough that the manuscript represented.

In 1990, Ljunggren and Kärre published in *Immunology Today* a very comprehensive review about the role of MHC class I Ags on NK cell recognition (2). According to the "missing self" hypothesis that they proposed, the absence or altered expression of MHC class I molecules would render target cells susceptible to NK cell attack. Klas Kärre adopted the concept of "missing self" while he was writing his Ph.D. thesis and found that it was easier to describe the common features of resistant target cells rather than susceptible ones (3). Four years earlier, in 1986, Kärre et al. published a seminal letter in *Nature* that showed in vivo that NK cells were able to reject tumor cells that have lost MHC class I expression (4). The finding that deficiency of MHC class I molecules constituted an alternative immune defense strategy was very provocative at the time. It was believed that NK cells worked like T cells by recognizing foreign Ags on the target cells, and it was very clear that NK cells were not MHC restricted. But what Kärre postulated was exactly the opposite in the sense that NK cells, like T cells, were strongly influenced by the expression of MHC class I molecules on the target cell (3). Following Kärre's letter in *Nature*, many manuscripts were published about the role of MHC class I molecules in NK cell recognition. In vivo and in vitro experiments were reported both in the mouse and in human systems. In general, people in the field accepted that NK cell susceptibility was directly related to the absence of MHC class I expression on target cells.

An important validation of the "missing self" hypothesis was the manuscript that showed a reversal of NK cell-mediated lysis of susceptible target cells that were transfected with DNA-encoding HLA class I molecules (5). Additional research showed that the protective effect of the HLA class I molecules mapped to the $\alpha 1/\alpha 2$ domains (6). Moreover, analysis by site-directed mutagenesis identified a residue in the peptide binding-groove that conferred susceptibility to NK cell lysis of HLA-A2-positive target cells (7). Further support for the "missing self" hypothesis was shown in manuscripts reporting that allogeneic bone marrow graft rejection could be prevented by expressing a H-2 transgene in donor mice (8), the rejection of class I MHC-deficient bone marrow cells by NK cells from MHC-matched mice (9), and the susceptibility of normal T cells from β_2-microglobulin deficient animals to normal NK cells (10). In addition, the phenomenon of hybrid resistance, where a $(A \times B)F_1$ host rejects A or B grafts (A and B refers to MHC genotype), was known to be dependent on NK cells. Moreover, the notion that the absence of self-MHC expression seems to target cells for rejection was a strong suggestion that susceptibility to NK cell lysis of the parental graft was, at least in part, linked to the absence of specific MHC class I products in the donor (8, 11). This observation supports the idea that NK cells recognize the absence of self. At the time, it also was known that human NK cells were a heterogeneous population, and they could be divided into subsets according to the expression of cell surface markers. NK clones with homogeneous expression for the specific receptors EB6 and GL183 were capable of allorecognition, and the sensitivity to NK lysis was mapped to the MHC and it was recessive (12, 13). It was also known that murine NK cells could be divided into two populations according to their reactivity with the 5E6 mAb and that $5E6^+$ and $5E6^-$ NK cells had different roles in the hybrid resistance phenomenon (11).

Two models were proposed to explain the role of MHC class I molecules controlling target cell resistance/susceptibility to NK cell lysis. The first model, the receptor inhibition model, stated that a putative receptor specific for MHC class I molecules on the NK cell will transmit an inhibitory signal that will turn off NK cell activation. The second model, the target interference model, postulated that ligands on target cells for activating NK cell receptors will be masked by the expression of MHC class I molecules, making them unable to trigger NK cell activation. Although there was some evidence supporting one model or the other, neither was conclusive at the time.

To validate the receptor inhibition model, it was important to identify the inhibitory receptor(s) expressed on NK cells that would block NK cell activation. In 1989, Yokoyama had cloned the receptor recognized by the A1 mAb from an EL4 expression library. He showed that the receptor was a type II transmembrane protein that belonged to the C-type lectin family and that this new receptor was a member of a family of highly related molecules (14, 15). Yokoyama was convinced that Ly49 (currently named Ly49A) must be involved in NK cell recognition (16). The fact that Ly49 belonged to a polymorphic family that was expressed on subsets of NK cells and the lack of knowledge

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regarding the molecular mechanisms involved in NK cell recognition were key factors for Yokoyama to focus his work on the role of Ly49 on NK cells (16). A logical step was to purify $Ly49^+$ NK cells and compare them with $Ly49^-$ NK cells. They observed that there were no phenotypic differences between the $Ly49^+$ and the $Ly49^-$ NK cells. They also observed that the classical NK cell target YAC-1 and other target cells were killed equally by both subsets. However, they found a considerable number of target cells that were not killed by the $Ly49^+$ NK cells while they were very susceptible to killing by the $Ly49^-$ subset. Moreover, the resistant target cells could not be lysed by other mechanisms, including Ab-dependent cellular cytotoxicity and redirected lysis, suggesting that a global block on NK cell activation was occurring. When they analyzed the mouse origin of the target cells, they found a correlation between the MHC class I expression on the target cells and their resistance/susceptibility to NK cell attack. Targets from $H\text{-}2^d$ and $H\text{-}2^k$ background were resistant to killing by $Ly49^+$ NK cells. Those results suggested that Ly49 is a receptor for an Ag of those haplotypes. This was the moment when Yokoyama made the connection with Kärre's "missing self" hypothesis (16). To demonstrate that specific MHC class I products were responsible for determining NK cell resistance by $Ly49^+$ NK cells, they transfected a susceptible cell line with cDNAs encoding several class I molecules. Only the one encoding for $H\text{-}2D^d$, but not $H\text{-}2K^d$ or $H\text{-}2L^d$, was able to confer resistance to killing by $Ly49^+$ NK cells, but not by $Ly49^-$ NK cells. To demonstrate further that there was an interaction between Ly49 and $H\text{-}2D^d$, they made cytotoxic assays in the presence of blocking Abs. The killing of resistant target cells by $Ly49^+$ NK cells was restored if mAb against Ly49 or the $\alpha1/\alpha2$ domains of D^d were present in the assay, whereas Abs against the $\alpha3$ domain could not restore the killing. Soon after Yokoyama's description of the first mouse NK inhibitory receptor, Moretta's group identified the first inhibitory receptors on human NK cells, the p58 (later named KIR2DL) molecules (17).

As a testimony to the relevance of his work, in 2001 Wayne Yokoyama received the Novartis Prize for Immunology, along with Klas Kärre and Lorenzo Moretta, for his description of the first NK cell inhibitory receptor specific for MHC class molecules. According to Rolf Zinkernagel, chairman of the selection committee, the three of them received the prize for "their scientific contributions. . . to our better understanding of the NK cell physiology and mechanisms of natural resistance against tumours and infections, not only conceptionally and experimentally, but also personally" (18).

Today, Yokoyama's lab is still producing magnificent and provocative papers, some of them discovering new roles for the Ly49 family of receptors. Two great examples are the identification of Ly49H as the activating receptor involved in the resistance to murine CMV infection (19) and the role of the Ly49 inhibitory receptors in the development of NK self-tolerance (20). We, the scientific community, and I, personally, owe Wayne much for his commitment and interest in doing good science and pursuing new questions and answers. Our intellect is well nourished with Yokoyama's contributions.

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