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NK cell recognition of unconventional ligands

Natural Killer (NK) cells are bone marrow derived lymphocytes capable of mediating rapid effector responses following pathogen detection in mammals[1]. The name "Natural Killer" derives from the ability of these cells to spontaneously lyse a large range of leukemia cell lines *in vitro* without prior sensitization[2 3]. NK cells evolved prior to adaptive lymphocytes and unlike B and T cells lack the ability to somatically rearrange antigen receptors. Thus it was hypothesized that NK cells are activated not by detection of foreign antigens, but failure to detect a common antigen expressed on all healthy cells, self/autologous class I major histocompatibility complex (MHC-I)[2 3]. The formation of this "missing-self" hypothesis was in part inspired by a biologically similar process that exists in Tunicates (Urochordata) whereby fusion of different colonies is dictated by a self-recognition system encoded by a single, highly polymorphic locus[2 3]. In mammalian NK cells, a highly polymorphic gene cluster encoding multiple inhibitory receptors for MHC-I, namely Ly49 genes in mice and KIR genes in man mediates self-MHC-I binding, preventing NK cell activation[4]. Genetic transformation of healthy tissue as well as viral infection is associated with reduced expression of MHC-I and is a proposed mechanism by which NK cells participate in the removal of such pathogenic cells *in vivo*[2 3]. While in most circumstances NK cells can be activated via "missing-self", the discovery that NK cells can also be activated following binding "altered-self" or "non-self" antigens via germ-line encoded receptors indicates that NK cells are more advanced than first thought. This ability of NK cells to recognize and respond to such unconventional ligands is the primary focus of a Special Feature review series in this issue of *Immunology and Cell Biology*.

NK cells are detected in all lymphoid organs and mature NK cells are found at a high frequency in the spleen, liver and peripheral blood. NK cells are short lived compared to adaptive lymphocytes and a steady production of immature NK cells in the bone marrow and their linear differentiation into two functionally distinct mature NK cell subsets in the periphery occurs[1]. This process critically relies on IL-15 trans-presentation by IL-15 receptor alpha (IL-15Rα) expressing myeloid and stromal cells[5]. In the article

by *Huntington* in this issue, the complex and unconventional mechanisms governing IL-15 expression and NK cell development are dissected in detail (XXX). During IL-15 mediated maturation, NK cells do not only increase their immune function (pro-inflammatory cytokine, perforin/granzyme production) but also gain expression of inhibitory self-MHC-I receptors (Ly49/KIR) presumably to prevent reactivity to self[1]. Ly49/KIR receptors capably of binding self-MHC-I associate with intracellular tyrosine based inhibitory motifs (ITIMs) and recruit phosphatases to guard NK cells in an inactive state. Interestingly, some members of the same receptor family have evolved to associate with adaptor proteins containing intracellular tyrosine based activation motifs (ITAMs) with ligand binding resulting in NK cell activation[6]. In the accompanying article by *Berry et al*, the authors detail the Ly49:MHC-I recognition system in mice and how this system has evolved to also actively recognize viral proteins, specifically those encoded by cytomegalovirus (XXX). Cytomegalovirus has evolved a number of immuno-evasion strategies including expression of proteins that mimic MHC-I. The authors discuss such strategies from a structural biology perspective and highlight the well described and intriguing evolution of the activating Ly49H receptor and its interaction with a cytomegalovirus encoded MHC-I-like protein that governs viral resistance in C57BL/6 mice.

In addition to encoding ligands to the activating Ly49/KIR receptors, several viral-encoded ligands have been shown to interact with members of the Natural Cytotoxicity Receptor (NCR) family. NCRs (NKp46, NKp44, NKp30) are activating type I transmembrane receptors of the immunoglobulin superfamily capable of binding ITAM containing adaptor proteins. Only NKp46 is conserved between human and mouse with this receptor being highly specific to NK cells in both species and used as a surface marker for the NK cell lineage. In the review article by *Kruse et al*, the authors highlight the recent identification of a range of viral, bacterial, parasitic and cellular-derived NCR ligands capable of activating NK cells through their respective NCRs and how NK cells contribute to the early immune response and inflammation in this context [7].

In arming themselves with a range of germ-line encoded receptors against foreign

antigens, NK cell have evolved be to a key mediator of the innate immune response to foreign pathogens, yet the original and arguably best described function of NK cells is the detection and killing of malignant cells, which in most cases is host-derived. Altered MHC-I expression is not the sole determinant for tumor killing by NK cells as NK cells unanimously express a two disulphide-linked type II transmembrane receptor, NKG2D, which binds self-antigens whose expression is induced during genetic stress such as that resulting in cancer[8]. In the review article by *Bert and Gasser*, the authors outline the current state of play in the NKG2D recognition of stress-induced ligands paying particular attention to the relationship between NKG2D ligand expression and cancer diagnosis and prognosis (XXX). The upregulation of NKG2D ligands on tumor cells, even in the presence of self MHC-I expression can result in NK cell mediated killing and suggests NK cell activation is determined by the net balance between inhibitory and activating receptor signaling. Furthermore, a family of adhesion molecules (DNAM-1, CD96 and TIGIT) that bind members of the nectin protein family has more recently emerged as important regulator of NK cell activity. DNAM-1, CD96 and TIGIT bind the ligands CD155 (Polivirus Receptor PVR; Necl5) and CD112 (Nectin-2, PRR2) and the review by *Ferrari de Andrade et al* summarizes the exciting new data on the synergy between activating receptor engagement and adhesion molecules in elucidating NK cell immune functions (XXX). Similarly to NKG2D ligands, DNAM-1 ligands are also expressed on a wide range of malignant cells and synergy between these molecules in NK cell activation and NK cell tumor immunosurveillance is also discussed.

As covered in multiple reviews in the current issue of *Immunology and Cell Biology*, adhesion molecules and activating receptors are central to NK cell recognition of their targets. This recognition is the first step in the formation of an immune synapse between NK cells and targets and involves cell tethering, adhesion and integrin signaling, nanotube formation and quantification of activating versus inhibitory signaling. The Recognition, Effector and Termination stages of NK cell cytotoxicity are central to tumor immunosurveillance and early control of viral infection and each coordinated step in these three stages is discussed with precision in the accompanying article by *Mace et al (XXX)*. Finally, while much attention has be given to NK cell recognition of ligands on

transformed or pathogen-infected cells, NK cells, like dendritic cells (DCs) express multiple pattern recognition receptors (PRRs) including members of the Toll-like receptors (TLRs) family capable of directly detecting viral and bacterial pattern-associated molecular patterns (PAMPs). DCs are sentinels of the immune system and upon detection of PAMPs, secrete a range of inflammatory mediators such as IFN-α/β, IP-10, MIP-1, IL-12, IL-15 and IL-18 that recruit NK cells and activate them independently or in a co-stimulatory fashion with ligands against activating receptors discussed in this issue's Special Feature[9]. Activated NK cells produce pro-inflammatory cytokines such as IFN-γ and TNF-α, which in turn promote DC maturation illustrating a complex synergistic relationship between innate myeloid and lymphoid cells during the initial phases of pathogen recognition[9]. In the review by *Adib-Conquy et al*., recognition of bacterial and viral PAMPs by NK cells and the role of TLR-mediated NK cell activation during infection are covered in detail [10].

Sadly, while this Special Feature of *Immunology and Cell Biology* was in preparation, Dr. Minou Adib-Conquy passed away after a long and courageous fight against cancer. We wish here to express our condolences to her family and her two sons and acknowledge her contributions to our field. The following tribute was contributed by Minou's colleagues Dr Fernando Souza-Fonseca Guimaraes (QIMR, Australia) and Jean-Marc Cavaillon (Institut Pasteur, France).

"Dr. Adib-Conquy joined Institut Pasteur in 1989 and bought her expertise in molecular biology to the group of Jean-Marc Cavaillon in 1997 where she began to ally both fundamental and translational research. Within the "Cytokines and Inflammation" laboratory she led her research team in deciphering the intracellular mechanisms governing functional changes in monocytes of sepsis patients or those suffering from non-infectious systemic inflammatory response syndrome. She rapidly acquired expertise in innate immunity and recently extended her investigations to the field of NK cells/TLRs and characterized the molecular mechanisms governing the prevention of endotoxin tolerance by IFN-γ and GM-CSF. Minou Adib-Conquy leaves us with the memory of a bright, intelligent, creative, rigorous and passionate scientist. She has been an extraordinary mentor, adored by her students for her kindness, her patience, her

availability, her dedication and scientific expertise. Her death is a great loss for the Institut Pasteur and the international scientific community."

In summary, the reviews included in this Special Feature of *Immunology and Cell Biology* highlight the multifaceted roles of NK cells in detecting unconventional ligands and countering a large range of pathogens and malignancies. The challenge ahead lies in harnessing NK cell function and advancing NK cell immunotherapy strategies to persistent infection, cancer and bone marrow transplantation.

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