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A KIR-TLR connection

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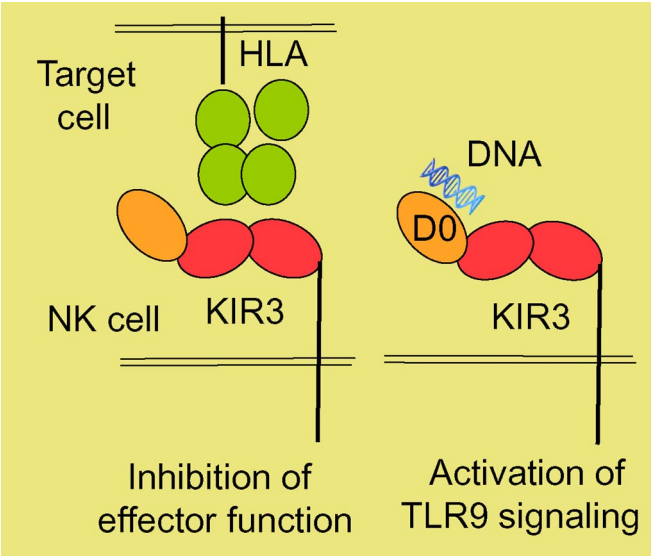
Killer immunoglobulin-like receptors (KIRs) are well-known for binding human leukocyte antigen (HLA) and allowing natural killer (NK) cells to monitor HLA levels on host cells. Unexpectedly, Sivori and colleagues have found that certain KIRs can also bind microbial nucleic acid and deliver it to endosome resident Toll-like receptors (TLRs), identifying a completely novel role for KIR family receptors.[1]



Diversity in KIR ligands and function KIRs inhibit NK cells effector functions upon the interaction with HLA molecules expressed on putative target cells. Using the membrane distal D0 domain, certain KIRs are now shown to bind DNA and activate TLR9 signaling in NK cells. Note that most KIRs are monomeric but that KIR3DL2 is actually a homo-dimeric receptor.

KIRs were originally discovered based on their ability to inhibit the effector functions of NK cells upon engaging specific HLA allotypes expressed on the cell surface of potential target cells. Consequently, inhibitory KIRs prevent NK cells from killing host cells that express HLA molecules at normal levels, allowing the specific detection of cells in which HLA expression is low. This so-called "missing-self" recognition represents an indirect recognition strategy used by NK cells to detect infected or transformed cells, which escape the direct antigen/HLA recognition by T cells. Besides the T-cell receptor, KIRs thus represent the prototype cell-surface receptor specific for HLA molecules. In a surprising twist, reported in this issue of *Blood*, Sivori et al find that certain KIRs can also directly bind unmethylated microbial cysteine guanine dinucleotide (CpG) DNA.[1]

KIRs are composed of 2 or 3 extracellular immunoglobulin (Ig) domains whereby HLA binding is mediated by the junction between the 2 membrane proximal Ig domains (see figure). The precise role of the third, membrane distal Ig-domain, the so-called D0 domain has remained enigmatic, even though it does play a role in HLA binding. This D0 domain in 3 Ig-domain KIRs, such as KIR3DL2, is now shown to directly bind CpG DNA (see figure). In contrast to HLA binding, KIR-mediated DNA recognition does not inhibit but rather stimulates NK cells to efficiently produce cytokines, such as Interferon-γ. Exactly how DNA binding induces KIR internalization rather than KIR clustering and inhibitory signaling is currently unknown. Irrespective of the mechanism, KIRs are shown to deliver DNA to endosomes where the acidic pH induces detachment and allows transfer to endosome resident TLR9. Even though other means exist to translocate DNA to endosomes, these new data suggest that certain KIRs permit human NK cells to directly recognize and efficiently respond to infection.

Mouse NK cells use a set of structurally distinct, but functionally equivalent Ly49 receptors for the recognition of major histocompatibility complex (MHC) class I molecules. A recurrent issue in the NK-cell field is whether human and mouse receptors perform equivalent functions. Here, despite the structural differences, one Ly49 family member, Ly49Q, may indeed perform a function similar, if not identical, to the one reported for KIR3DL2. Ly49Q binds certain MHC class I allotypes[2] and was recently reported to play an important role in TLR9 signaling. Rather than playing a role in NK cells, Ly49Q is important for efficient TLR9-mediated production of type I Interferon by plasmacytoid dendritic cells.[3] Even though it is not known whether Ly49Q binds DNA, the data provide intriguing evidence that structurally distinct human and murine MHC class I receptors are involved in TLR signaling.

What is the significance of the KIR-TLR connection in health and/or disease? The new

data raise the possibility that immune cells co-expressing specific KIR and TLR9, such as NK cells and a subset of T cells, play a prominent role in directly recognizing and responding to pathogens or to host cell death. As one possible pathologic role, KIR3DL2 is selectively expressed in Sézary syndrome,[4] a cutaneous T-cell lymphoma, which is associated with skin-related infections. Sivori et al speculate that the tumorigenic process may be initiated by infection, which chronically triggers KIR3DL2 mediated T-cell proliferation.

Finally, multiple genetic studies have shown an association of inhibitory KIR with diseases as diverse as infection, cancer, and autoimmunity. In most cases, the strongest influence on disease outcome is due to *KIR-HLA* gene interactions.[5] One study showed a significant correlation between carriers of a particular *KIR3DL2* allele and the induction of an efficient NK-cell response to *Plasmodium falciparum*–infected erythroctyes.[6] Because erythrocytes lack HLA molecules and based on the new findings reported by Sivori et al, it is tempting to speculate that the recognition of DNA causally linked the expression of KIR3DL2 to NK-cell activation, perhaps providing the first evidence for the benefits of KIR-TLR connection.

Conflict-of-interest disclosure: The author declares no competing financial interests. ■

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Sialic acid–IVIg targeting CD22

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In this issue of *Blood*, Séïté and colleagues report their findings on human tonsil B cells and human Ramos B-cell lymphoma line apoptosis, induced by sialic acid–intravenous immunoglobulin (SA-IVIg) fraction.[1]

IVIg is used for treatment of many inflammatory, autoimmune, and immune-deficiency disorders (reviewed in Tha-In et al,[2] Schwartz-Albiez et al,[3] and Nimmerjahn and Ravetch[4]). Because IVIg contains antibodies (Abs) toward idiotypes (Ids) specific for autoAbs, it modulates autoreactive B cells. Several mechanisms have been proposed to explain IVIg's activity, including blocking of phagocytic Fcγ receptors (Fcγ), up-regulation of inhibitory FcγR (FcγRIIb), modulating cytokine secretion, inhibiting cell proliferation, priming dendritic cell regulatory activity, and induction of T regulatory cells.[2-4] B lymphocytes harbor IVIg-targeted molecules that set up the bone marrow B-cell repertoires, negative signaling through FcγRIIb, selective down-regulation of Ab production, and neutralization of circulating autoAbs by anti-Ids. Anti-Id–specific IVIg was found to be an efficient approach in treating animal models of human autoimmune diseases, such as systemic lupus erythematosus (SLE), antiphospholipid syndrome, and myasthenia gravis (MG).[5,6] Manipulation of B cells by IVIg is not only passive (with neutralization of autoAbs and FcγR blockade) but also active (with effect on B-cell antigen receptor [BCR] signaling). This is regulated either positively or negatively by various B-cell membrane molecules (see figure).

BCR ligation regulates B cells from an immunoreceptor tyrosine-based activation motif (ITAM) through a cascade of kinase phosphorylation. Signals initiated at the BCR regulate transcriptional, posttranscriptional, and posttranslational events, determining the fate of the B cell—whether it will survive, die, proliferate, or differentiate. Regulation of BCR signaling is mediated by the association of the BCR complex with coreceptors such as CD22, CD19, CD21, and FcγRIIB.

CD22 is a transmembrane adhesion molecule that belongs to the sialic acid (SA)–binding Ig-like lectin (Siglec) superfamily, with 7 Ig-like extracellular domains and an amino-terminal Ig domain. CD22 modulates the BCR signaling cascade by binding SA-modified glycoproteins. SA binding to CD22 is required for negative regulation of BCR signaling. Upon stimulation of the BCR, the cytoplasmic tail of CD22 is phosphorylated on tyrosine residues of Ig-like tyrosine-based inhibitory motifs (ITIMs). Different intracellular signaling proteins bind to the phosphorylated tyrosines of the CD22 tail. BCR signaling is inhibited through recruitment of the Src homology 2 domain-containing phosphatase-1 protein tyrosine phosphatase via ITIMs in the CD22 cytoplasmic tail, followed by the dephosphorylation cascade of other signal proteins.

Séïté et al, in the current issue, used SA-IVIg as a ligand molecule for CD22. The SA-enriched IVIg fraction, which is an IVIg-glycan specific for CD22, was affinity-purified from commercial IVIg using a *Sambucus nigra* agglutinin agarose column.[1] The SA-IVIg was used to prove that B-cell membranous CD22 is a key receptor in IVIg-mediated BCR signaling. SA-IVIg through IVIg-CD22 promotes apoptosis in mature human tonsil B lymphocytes and in human Ramos lymphoma B-line cells. The IVIg-CD22 association involves several BCR-signaling pathways including inhibition of the phospholipase Cγ2 cascade, sustained activation of Erk1/2, p38, and down-regulation of PI3K. These changes are associated with the induction of cyclin-dependent kinase inhibitor $p27^{Kip1}$, which inhibits cell-cycle progression at the G1 phase and thus promotes apoptosis.

B cell–depleted therapy by SA-IVIg has a strong potential for treating systemic and organ-specific autoimmune diseases including SLE, MG, multiple sclerosis, and other autoimmune conditions where their activity is mediated by BCR cell function. The efficacy of SA-IVIg was previously reported by Ravetch et al to be involved in the treatment inflammatory state.[7] They demonstrated that the anti-inflammatory activity of IVIg is mainly mediated by Abs that harbor terminal α2,6-sialic