TIGIT: A Novel Entry in NK Cell Regulation

By Sophia Khatri

While TIGIT's immunoregulatory role in T Cells has been described, the mechanisms through which TIGIT modulates NK Cell activity has remained elusive. Recent research by Staniesky et al. reveals that the expression of TIGIT by NK cells inhibits the lysis of PVR and PVRL1-expressing target cells in an ITIM-dependent manner.

The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity by Stanietsky et al. confirms the T cell immunoreceptor with Ig and immunoreceptor tyrosine-based inhibitory motif (ITIM) domains (TIGIT) as a functioning receptor that plays a critical role in limiting adaptive and innate immunity in NK cells.[1,2] In recent years, TIGIT has been suggested to modulate NK and T cell activity as it is expressed on both T cells and NK cells.[1] Previous research has evaluated TIGIT's role on T cell activity and found that it indirectly inhibits T cells through the manipulation of DC activity.[1] While its role on T cells was being clarified, little work was done to show its function on NK Cells. However, if TIGIT suppressed NK activity similar to what was seen on T cells, this would expand the therapeutic potential of targeting this receptor. However, learning more about TIGIT, specifically its function on NK cells, its different ligands, its mechanism of action, and how blocking antibodies could be used to prevent its activation, was essential.

While T cells have a single dominant antigen receptor, NK cells possess a variety of receptors, TIGIT being one of these. TIGIT is a transmembrane co-receptor comprising an immunoglobulin variable, a transmembrane, and ITIM domains.[2] Previous research has demonstrated that the Poliovirus receptor (PVR) and PVR-like family members have a high affinity for this co-receptor.[1] However, that work only evaluated the interaction of PVR-like molecules, their interaction with TIGIT, and how they affect T cell activity. Further, TIGIT's activity on NK cells was expected to be minimal because another PVR-binding molecule, CD226, is expressed at a much higher level than TIGIT and was expected to outcompete for binding. Stanietsky et al. set out to evaluate the interaction between TIGIT and PVR-like proteins specifically on NK cells.[2]

In this work, human YTS NK cells transduced with TIGIT (YTS/TIGIT) were used as a model line to evaluate TIGIT's role in controlling NK activity. 721.122-Human Leukocyte Antigen-negative B cells and those expressing PVR (721.122/PVR) were tested for cell lysis by YTS/TIGIT cells. This was done to evaluate the specific interaction between TIGIT and PVR. YTS/TIGIT cells were then pre-incubated with an anti-TIGIT antibody to block the interaction between PVR and TIGIT showing its effect on NK-mediated lysis. It was found that there was a significant increase in NK activity resulting in increased lysis of the 721.122/PVR cells compared with nontransfected cells. This was consistent with an inhibitory role for TIGIT on NK activity. This finding coincided with research by Yu et al. on TIGIT's immunosuppressive role.[1]

Given an inhibitory role for TIGIT, it was necessary to elucidate a mechanism for this inhibition. It was suggested that an ITIM motif was crucial for this inhibitory activity.[3,4] In order to test this, Stanietsky et al. created two modified TIGIT genes, one with a truncated receptor, bearing an interrupted ITIM motif, referred to as Y231stop, and a second bearing a point mutation within the ITIM motif referred to as Y231A. These altered TIGIT proteins were expressed in YTS cells alongside control-transfected YTS cells expressing either the full-length protein or only a GFP marker. Cells expressing the altered proteins were then evaluated as effectors of the lysis of 721.221 vs 721.221 PVR cells. It was concluded that there was no significant difference in the percentage of lysis of the 721.221 and 721.221/PVR cells when treated with YTS / GFP, YTS/Y231stop, and YTS/Y231A cells. However, when these cells were co-cultured with YTS/TIGIT cells, a significant decrease in cell lysis of 721.221/PVR cells was observed suggesting that TIGIT's inhibitory activity on NK cells relies on its ITIM.

After learning about TIGIT's role in NK activity and what specifically controls its suppressive nature, Stanietsky et al. evaluated other PVR-like proteins. Previous research had demonstrated that, like PVR, PVRL2 and PVRL3 also had a high affinity to TIGIT1. To test the different PVR-like proteins, PVRL2 and PVRL3 were transfected into 721.122 cells. The affinity of DNAM1-Ig and TIGIT-Ig was assessed on the PVR-like-expressing cells by flow cytometry revealed that both DNAM1-Ig and TIGIT-Ig had a strong affinity to PVR, lesser affinity to PVRL2, and, surprisingly, no significant affinity to PVRL3, which contrasted with Yu et al.'s previous findings. Once the affinity of TIGIT for PVR-like proteins was established, the function of YTS/TIGIT and YTS/TIGIT Y231stop cells' ability to lyse PVRL1- and PVRL2-transfected 721.122 cells was assessed. It was concluded that, consistent with their relative affinities, PVR showed strong inhibition of cell lysis, PVRL2 was less efficient in inhibiting lysis, and PVRL3 exhibited no inhibition of lysis at all. The YTS/TIGIT Y231stop cells were used to further demonstrate that the inhibition is ITIM-dependent, as there was no NK inhibition when this domain was interrupted. Altogether, this work demonstrated that when TIGIT is expressed on human NK cells, it inhibits the lysis of PVR and PVRL1-expressing target cells and that TIGIT's inhibitory activity on NK cytotoxicity is entirely dependent on the ITIM domain. Finally, it was established that TIGIT has a higher affinity for PVR relative to other PVR-like proteins and that inhibition of lysis correlates with this affinity. TIGIT, therefore, acts as a vital immunomodulator protein, with the ability to regulate the activity of both NK cells and T cells. These findings all point toward a potential clinical role for TIGIT-targeting immunotherapies.

References

1. Yu, X. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Novemb. 16 2008.

2. Stanietsky, N. The interaction of TIGIT with PVR and PVRL2 inhibits human NK cell cytotoxicity. Sept. 1 2009.
3. Maeda, A. Paired immunoglobulin-like receptor B (PIR-B) inhibits BCR-induced activation of Syk and Btk by SHP-1. April 8 1999 doi:10.1038/sj.onc.1202552.
4. Muta, T. A 13-amino-acid motif in the cytoplasmic domain of Fc gamma RIIB modulates B-cell receptor signalling. 1994 Mar 3 doi:10.1038/368070a0.