# HLA-E and HLA-G Co-expression in Porcine Endothelial Cells Attenuates Human Natural Killer Cells degranulation

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**Background:**
Xenotransplantation offers a prospective solution to organ shortage. The elimination of major xenoantigens in pig cells prevents hyperacute xenograft rejection (HXR), driven by preformed antibodies. However, acute xenograft rejection (AXR), driven by immune cell activation, continues to be a barrier in pig-to-human xenotransplantation. Natural killer (NK) cells, with inhibitory and activating receptors, play unique roles in AXR by promoting graft rejection or tolerance. NK cell tolerance occurs naturally in utero where human leukocyte antigen (HLA)-E and HLA-G are present. Expressing HLA-E/G in xenografts may provide immune protection from human NK cell cytotoxicity.

**Methods:**
This study aims to demonstrate the use of HLA class I molecules in inducing human NK cell tolerance. 5-gene-knock-out (5GKO) porcine endothelial cells (pECs) were transfected with HLA-E and HLA-G genes. Transfected cells were stained with HLA-E and/or HLA-G antibody. HLA class I expressing 5GKO cells were isolated by flow sorter. Genetically modified pECs were co-cultured with human peripheral blood mononuclear cells (PBMCs) for E:T ratio of 10:1 for 2 hours. PBMCs were collected and stained with fluorochrome-conjugated antibodies. NK cell degranulation was accessed by the percentage of CD107a expression in the $CD3^{-}CD56^{+}$ population. Co-localization of HLA-E and HLA-G on pECs was imaged by immunofluorescence microscopy and quantified by Image-Pro.

**Results:**
5GKO pEC lines expressing HLA-E, HLA-G, and co-expressing HLA-E and HLA-G were successfully established. Co-expression of HLA-E and HLA-G in 5GKO pECs reduced human NK cell degranulation by 50% compared to the 21% reduction achieved with 5GKO alone ($p<0.0001$). Co-localization intersection of 5GKO.HLA-E.HLA-G with stimulated PBMCs was not significantly different than when cultured with media (0.067 vs. 0.059, $p=0.57$).

**Conclusion and Impacts:**
Co-expression of HLA-E and HLA-G in 5GKO pECs significantly reduced human NK cell degranulation, compared to 5GKO.HLA-E, 5GKO.HLA-G, and 5GKO pECs. However, co-localization of HLA-E and HLA-G didn't change when cultured with stimulated PBMCs. This study provides insight into the interactions between HLA molecules that promote an immunotolerant phenotype.