# beta2-Microglobulin-free HLA-G activates natural killer cells by increasing cytotoxicity and proinflammatory cytokine production.

**Journal:** Hum Immunol

**Publication Year:** 2013

**Authors:** Longmei Zhao, Bhamini Purandare, Jian Zhang, Basil M Hantash

**PubMed link:** 23228393

**Funding Grants:** San Jose State University Stem Cell Internships for Laboratory-based Learning (SJSU SCILL)

**Public Summary:**
Human leukocyte antigen-G (HLA-G) is a nonclassical HLA class-I molecule and plays a role in tissue specific immunoregulation. Many studies have addressed functional aspects of beta2-microglobulin (beta2m)-associated HLA-G1. beta2m-free HLA-G has been found in human placental cytotrophoblasts and pancreatic beta cells although its function remains unclear. In the present study, we investigated the function of beta2m-free HLA-G by transfecting HLA-G1 and -G3 into human beta2m deficient rat pancreatic beta cell carcinoma (BRIN-BD11) cells. RT-PCR and western blots studies confirmed high expression of HLA-G1 and -G3 in -G1 and -G3 transfectants, respectively. HLA-G1 and -G3 were detected mainly in intracellular compartments of BRIN-BD11 transductants by confocal fluorescent microscopy and flow cytometry. Functional analysis revealed that beta2m-free HLA-G promoted xenogeneic cytotoxic lysis of BRIN-BD11 cells by natural killer (NK) cells and increased production of IL-1beta, TNF-alpha, and IFN-gamma. Stimulation of cytotoxic lysis was impaired by blocking the MAPK and DNA-PKcs pathways in NK cells. Importantly, treatment with 33mAb, a KLR2DL4 receptor agonist, induced NK-mediated cytotoxic lysis of BRIN-BD11 cells transfected with a mock vector. Our data suggest that beta2m-free HLA-G activates NK cells via engagement of KLR2DL4 receptors.

**Scientific Abstract:**
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