ImmunoTools *special* Award 2015

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Functional impact of KIR3DL2 polymorphism in pemphigus

Background

Pemphigus is a group of autoimmune blistering disease that affects skin and mucous membranes. It is an autoantibody-mediated disease characterized by antibodies against desmogleins (Dsg 1 and Dsg3). Two major pemphigus subtypes differ according the clinical and histological features and autoantibodies targets: pemphigus vulgaris (PV) is mediated by autoantibodies against desmoglein (Dsg) 1 and 3 and pemphigus foliaceous (PF) by anti-Dsg1 (Denning et al.,1998; Anhalt et al., 1982).

Pemphigus vulgaris has a broad distribution around the world; pemphigus foliaceus has an endemic form, known as Fogo Selvagem (FS) and it occurs in some areas of Brazil and in neighbouring South American countries (Gonzalez et al., 2006; Ortega et al., 2013; Robledo et al., 1988). The etiology of PF is still unknown but several genes of immune response have been associated with this disease.

Natural killer (NK) cells are key components of the innate immune response (Pierre *et al.,* 2010). Human NK cells comprise approximately 15% of all lymphocytes (Nagler *et al.,* 1990). Although NK cells are considered traditionally part of innate immunity they are also important for adaptive immunity including autoimmunity.

Killer-cell immunoglobulin-like receptors (KIR) are expressed on the surface of NK cells and subpopulations of activated or memory T cells (Parham *et al.,* 1997). These receptors recognize HLA class I molecules that are on the surface of majority of cells, regulating the balance of activating and inhibitory signals that modulate NK

cell response. KIR genes exhibit uncommon presence/absence polymorphisms that combined with allelic variation make difficult to characterize the diversity of this gene family.

KIR3DL2 is a framework gene and is present virtually in all haplotypes, consequently in all individuals. It is also the second most polymorphic KIR, with more than 80 alleles described. In addition, this receptor is expressed in higher levels on the surface of NK comparing to other KIR. KIR3DL2 binds HLA-A*03/A*11 *in vitro* (Hansasuta et al., *2004)* and there are evidences that these ligands are also recognized *in vivo* (Augusto et al., submitted).

It has been shown that activating KIR genes are associated with increased risk of autoimmunity. However, particularities of endemic PF, like the well-documented influence the environmental exposure in the pathogenesis, may be the reason why activating KIR are protecting against PF (Augusto et al., 2012). *KIR3DL2*001* confers risk to pemphigus susceptibility what suggests that this allotype is stronger inhibitory than other KIR3DL2 (Augusto et al., submitted). Previous and unpublished results from our group suggest that NK cells from *KIR3DL2*001*$^{+}$ *KIR3DL1*$^{+}$ individuals were more efficiently inhibited than cells presenting other KIR phenotypes when co-cultured with T cells from *HLA-A*03/A*11 HLA-Bw4/Bw4* individuals. However these results are not conclusive and the functional impact of KIR3DL2 in pemphigus is not elucidated.

Rationale

We demonstrated that activating KIR have been associated with protection against PF and KIR3DL2*001 is a risk factor. We hypothesized that KIR3DL2*001 is stronger inhibitory than other KIR3DL2 and variation in KIR3DL2 contributes significantly to increased risk of developing pemphigus. This is probably caused by differential binding ability and/or signal transduction of different allotypes.

Aims

To formally demonstrate that KIR3DL2*001 has a stronger inhibitory potential than other allotypes;

To test the inhibitory potential of different alleles;

To characterize the influence of the KIR and HLA genotype on the level of cytotoxicity by NK cells from healthy individuals and patients *in vitro*.

Methods

NK and T cells isolation from PBMC.

Flow cytometry to evaluate cell purity and to analyze expression of different KIR3DL2 allotypes on the cell surface.

Co-culture of T cells and NK cells with specific KIR/HLA genotypes.

Cytotoxicity and cytokines detection assays