ImmunoTools *special* Award 2014

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The effects of ERAP1 polymorphisms on the peptide repertoire in HLA-B27 positive individuals

HLA-B27 is strongly associated with Ankylosing Spondylitis (AS); although its presence predisposes to AS, on its own it is insufficient to cause the disease (1). Genome-wide association studies (GWAS) have confirmed the polygenic nature of AS and identified other potential susceptibility genes, such the endoplasmic reticulum aminopeptidase 1 (ERAP1) (2). ERAP1 is critical for the generation of many antigenic epitopes in vivo and can influence the generation of the antigenic peptide repertoire. Interestingly, the ERAP1 disease association with AS is restricted to B27-positive cases. Of the known ERAP1 single nucleotide polymorphisms (SNPs), two have been repeatedly confirmed to confer strong susceptibility to the disease: rs27044 C/G and rs30187 C/T. Notably, the protective allelic variants of ERAP1 show a reduced rate of peptide-trimming activity, suggesting that a loss of function could be beneficial for the disease (3-4).

Here, I want to verify the influence of the two SNPs in the ERAP1 gene known to be associated with AS on the processing, the stability and the peptide antigen repertoire of HLA-B27 molecules.

Briefly, I will isolate PBMC from blood samples of HLA-B27 positive individuals (either AS patients or controls), and I will select T CD8+ sub-population; the purity of this population will be assessed by flow cytometry using a combination of CD3-CD8 and CD3-CD4 antibody. $CD8^+$ T cells will be cultured in an appropriate medium supplemented with rh-IL2 and stimulated in an antigen-specific manner using viral peptides derived from EBV (pEBNA3C, pLMP2). To verify the activation of $CD8^+$ T cells after stimulation, I will perform a membrane staining of the main activation markers such as CD25, CD40, CD69, HLA-ABC and HLA-DR; as control for the activation, $CD8^+$ T cells will be incubated with rh-IFN-γ.

By using chimeric proteins as antigen suppliers, that cross the cell membranes by virtue of the TAT transducing domain from HIV, my group has disclosed an alternative route of antigen processing and cross-presentation that is operating in the case of epitopes restricted by HLA-B27 but not for those restricted by HLA-A2 molecules (5). This pathway is proteasome-, TAP-, and APC-independent and

requires that the chimeric proteins reach the TGN where the antigenic epitopes are loaded by the B27 molecules. These findings allow to speculate that the TGN, endowed with a relative acidic environment, represents, for some MHC class I molecules which can leave the ER in unstable conditions, a cell compartment where to exchange peptides that, in certain circumstances, i.e. ingress of exogenous proteins into the secretory vesicles, fill the groove of these metastable molecules thus rescuing them from degradation. TAT chimeric proteins, including pLMP2 or pEBNA3C peptides, will be transduced in B-LCLs having all different rs27044/rs30187 haplotypes and these B-LCLs will be used as target cells in cytotoxic assays in which pLMP2 (or pEBNA3C)-specific and HLA-B27-restricted $CD8^+$ T cells will be used as effector cells. The rationale is that the efficiency of ERAP1 to trim the peptides should determine the amount of HLA-B27 molecules that would reach the TGN in a stable (optimal peptide length) or unstable (suboptimal peptide length) conformation and, in the latter case, prone to exchange peptides. If so, a minor enzyme activity of ERAP1 could be related to a better recognition by CTLs of TAT chimeric proteins, including pLMP2 or pEBNA3C peptides, that once in the TGN could replace the endogenous 'suboptimal' peptides and loaded by HLA-B27 molecules.

Variation in the ERAP1 activity can affect the peptide binding repertoire displayed by HLA-B27 molecules so the ERAP1-peptide-HLA-B27 functional interaction could be the missing link in the pathogenesis of AS.

References

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