Titolo della ricerca: How the interplay between HLA-B27 and other risk genetic factors sets the CD8+ T cell responses in Ankylosing Spondylitis and viral immune surveillance

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DESCRIZIONE DELLA RICERCA

The HLA (human leukocyte antigen)-B27 class I gene represents the strongest risk factor for the Ankylosing Spondylitis (AS), a chronic rheumatic inflammatory disease belonging to the group of Spondyloarthritis (1,2). The main role of HLA-B27 is to present antigenic peptides to the CD8+ T cells but its pathogenic function in the disease is still unclear (1). Although animal models do not support a direct involvement of CD8+ T cells in the AS pathogenesis, GWAS (Genome Wide Association Studies) have identified, as risk factors, several genes implicated in antigen processing (Endoplasmic Reticulum resident aminopeptidases, ERAP1 and ERAP2) and in the T cell development and function (RUNX3, TBX21, IL7R, EOMES and ZMIZ1), thus refocusing the attention on these cells (3-5). In particular, ERAP1 and 2 influence the antigenic presentation as they cut the microbial and autologous peptides at the N-terminal end to an optimal length for loading by class I HLA molecules. On the other hand, the main function of the HLA-B27 molecules is to present the viral and heterologous peptides to the cytotoxic CD8+ T lymphocytes. This demonstrates the convergence of these risk factors on the same molecular pathway and candidates the peptide repertoire as a key element in AS pathogenesis. Therefore, it is legitimate to hypothesize an involvement of CD8+ T cells in AS (6). Importantly, the HLA-B27 besides predisposing to AS, and more generally to Spondyloarthritis, also gives a more efficient protection against viral infections (HIV, HCV, EBV and influenza virus) thanks to a superior "performance" of HLA-B27-restricted, cytotoxic T cells (7,8). So, this better viral immunosurveillance linked to the HLA-B27 could have as downside the association to autoimmunity (7,8). Therefore, the aim of this project will be to characterize
the cytotoxic CD8+ T cell in patients with AS taking also into account the ERAP1 and 2 genotype (4). Several aspects related to the functional and metabolic characteristics of CD8+ T lymphocytes will be analyzed. In particular, the research will focus on the following points:

1. **Analysis of the presentation of atypical antigenic peptides.** For many years, our group has been studying the atypical presentation of viral peptides and self peptides by the HLA-B27 molecules using as an experimental model, the comparison between the B*2705 strongly AS associated, and the B*2709 allele. The latter does not predispose to AS and differs from the first one only for the His116Asp polymorphism that influences many of the functions of these two molecules (9). Studies are in progress on the presentation of viral peptides that do not have the optimal B27 binding motif and therefore must assume anomalous conformations (10). It is interesting that these peptides are presented by the B*2705 but not by the B*2709 molecules (10). These studies are performed in AS patients and controls taking into account the polymorphisms of ERAP1 and 2 that influence the peptide repertoire.

Furthermore, in collaboration with Prof. M. D'Abramo, the possible conformations assumed by these peptides when associated with HLA-B27 molecules will be evaluated through molecular dynamics simulations and molecular "modeling".

2. **Analysis of lymphocyte migration induced by chemokine gradient.** These experiments will be performed both on CD8+ T lymphocyte cell lines specific for viral antigens and on CD8+ T populations *in toto* isolated *ex vivo* from HLA-B27 positive and negative patients with AS compared to healthy controls. Migration to a chemokine panel (CXCL9, CXCL10, CXCL11, CXCL12, CCL20) and expression of related receptors will be evaluated. Preliminary results suggest that CD8+ T cells from patients have a greater intrinsic and chemo-independent migratory capacity than cells of healthy subjects. To investigate this point, the chemokines present in the plasma from patients will be analysed.

Experiments will be also carried out to analyze the level of actin polymerization and the activation status of the Rho GTPases and coflin important for leukocyte trafficking (11). We also want to correlate the migration capacities to senescence and exhaustion phenotype imposed by the chronic inflammation through the measurement of lymphocyte telomere length (12).

3. **Characterization of the metabolic pathways used during effector phases.** The increased efficiency of CD8+ HLA-B27-restricted T lymphocytes could be due to a better use of nutrients and an optimization of the production of energy that are required during
the effector phases of the antiviral response. In collaboration with the group of Dr. Battistini who has the appropriate facility (Seahorse Bioscience), we intend to evaluate the metabolic phenotype of cells in relation to the use of glycolysis (measurement of ECAR extracellular acidification rate) and mitochondrial respiration OXPHOS (measurement of OCR oxygen consumption rate) following cell activation (13). The ability of these cells to function under conditions of increased energy demand will also be evaluated. Moreover, the metabolic requirements for CD8+ T cell activation and expansion will be also assessed by using several inhibitors of specific metabolic pathways (14).

Referenze


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