DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

Proposta di assegnazione di una borsa di Dottorato

Titolo della ricerca:
Effects of the interplay between HLA-B27 and Endoplasmic Reticulum Aminopeptidases (ERAP1 and 2) on CD8+ T cell responses in autoimmunity and antiviral defense

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DESCRIZIONE DELLA RICERCA (max 2 pagine)

Obiettivi della ricerca e stato delle conoscenze

The HLA (human leukocyte antigen) class I gene, HLA-B27 has been known for many years as the major risk factor for Ankylosing Spondylitis (AS), a chronic inflammatory and autoimmune rheumatic disease (1,2). However, the pathogenic role played by this factor in the disease is still far from a complete definition (2). Recently, the GWAS (genome wide association studies) allowed to identify other susceptibility factors for the AS including Endoplasmic Reticulum AminoPepidases, ERAP1 and 2 (3,4). These aminopeptidases 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. It is very interesting that between HLA-B27 and ERAP1 there is an epistatic interaction; indeed, the association between AS and ERAP1 is only found in patients who are HLA-B27 positive. This demonstrates the convergence of the two risk factors on the same molecular pathway and highlights the peptide repertoire as a key element in the pathogenesis of AS. Therefore, considering that the main function of the HLA-B27 molecules is to present the viral and heterologous peptides to the cytotoxic CD8+ T lymphocytes, it is legitimate to hypothesize that this cellular population has a pathogenic function in the AS (5). Another important observation is that the HLA-B27 in addition to predispose to AS, and more generally to Spondyloarthopathies, 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 (6,7). The reasons are not entirely known and may be of a "virological" and "immunological" nature. So, this positive aspect of the acquired immunity linked to the HLA-B27 could have as downside the association to autoimmunity (6,7). 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 (4) genotype. Several aspects related to the functional and metabolic characteristics of CD8+ T lymphocytes will be analyzed.

In particular, the research will be developed in 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 allele that is associated with the disease and the B*2709 allele that does not predisposes to the pathology and differs from the first only for the His116Asp polymorphism that influences many of the functions of the two molecules (8). 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 (9). It is interesting that these peptides are presented by the allele B*2705 associated with the AS but not by the B*2709 (9). These studies are performed in AS patients and controls taking into account the polymorphisms of ERAP1 and 2 that influence the peptide repertoire and their relative amount. 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 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 chemoindependent migratory capacity than cells of healthy subjects. To investigate this point, the chemokines present in the patient plasma will be analyzed and furthermore, tests will be carried out to analyze the level of actin polymerization and the activation status of the Rho GTPases and cofilin important for leukocyte trafficking (10). 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 (11).

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 (12). The ability of these cells to function under conditions of increased energy demand will also be evaluated.

Referenze


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