DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

Proposta di progetto per una borsa Dottorato Sapienza
Linea di ricerca secondaria

Titolo della ricerca: Role of CD28 and associated class 1A PI3K in the regulation of IL-22-producing CD4^{+} T cells in Multiple Sclerosis.

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DESCRIZIONE DELLA RICERCA *(max 2 pagine, Arial 12, interlinea singola, esclusa bibliografia)*

Obiettivi della ricerca (generale e specifici)

The present project will be aimed to characterize the role of CD28 and associated class 1A PI3K in the regulation of IL-22 expression and IL-22-producing T cell subset amplification in Multiple Sclerosis (MS).

To reach this goal we will proceed with the following tasks:

Task 1. Role of CD28 and associated class 1A PI3K in the regulation of IL-22 production and IL-22-producing T cell amplification in MS patients in different phases of the disease.

Task 2. Characterization of the signalling pathways and transcription factors regulating CD28-mediated transcription and secretion of IL-22 in MS.

Task 3. Functional relevance of CD28-mediated IL-22 production and IL-22-producing cell amplification in astrocyte and oligodendrocyte functions.

The results that we plan to obtain from the present project will contribute to a better insight into the pathophysiological mechanisms of MS and will allow the identification of IL-22-producing inflammatory T cells as new targets for class 1 PI3K-based therapies in MS.

Stato delle conoscenze

Pro-inflammatory T lymphocytes play a critical role in the pathophysiology of MS. The cytokine- and chemokine-producing phenotype of self-reactive T cells in MS patients determines the ability of these cells to cross the blood-brain-barrier and cause inflammation in the central nervous system (CNS), thus contributing to disease progression. For instance, conversion of MS from the relapsing-remitting (RR) to progressive phases has been related to prolonged chronic inflammation in the CNS. Emerging data indicates that IL-22 and IL-22-producing T cells may contribute to MS pathogenesis by initiating the autoimmune response against CNS myelin. However, the role of IL-22 and IL-22 producing cells in MS has not yet been completely elucidated as well as the receptors and signalling molecules regulating IL-22 expression.

CD28 may be considered one of the most important costimulatory receptor necessary for full T cell activation and for preventing anergy. By binding its cognate ligands B7.1/CD80 or B7.2/CD86 on the surface of professional antigen presenting cells (APCs), CD28 lowers TCR activation threshold and leads to the augmentation of early signalling events necessary for efficient cytokine production, cell cycle progression and survival (1). The role of CD28 in MS pathogenesis has been extensively studied in animal models. Initial studies suggested that CD28/B7 interaction is essential for the development of EAE (2). However, data from Vogel et al evidenced that the blockade of B7 by CTLA4-Ig or anti-B7 mAbs after T cell priming led to severe CNS inflammation and demyelination and exacerbated EAE (3). Furthermore, recent data from a randomized clinical trial of abatacept did not evidence any significant efficacy in reducing neuroinflammation in

relapsing-remitting RRMS patients (4). These discrepancies may be related to the ability of CTLA-4Ig to inhibit both costimulatory signalling through CD28 and co-suppressive signals mediated by CTLA-4 (5). More recent data by Haanstra et al. showing the reduction of both CNS inflammation and demyelination in human EAE in rhesus macaques following administration of FR104 CD28 blocking Ab (6), strongly support a crucial role of CD28 in regulating the expansion and inflammatory function of autoreactive T cells in MS. Finally, the identification of single nucleotide polymorphisms (SNPs) within genes belonging to CD28/CTLA-4/CD80/CD86 pathway as risk genes for MS, highlights the relevance of costimulation in MS pathogenesis (7).

In the human system, CD28 is also able to emanate TCR-independent autonomous signals regulating pro-inflammatory cytokine/chemokine production (8). We have recently demonstrated that human CD28 stimulation by agonistic antibodies (Abs) or B7 expressed on APCs, in the absence of TCR engagement, is able to activate a specific NF-κB pathway in peripheral $CD4^+$ T cells, leading to the production of pro-inflammatory cytokine/chemokines (9), in particular cytokines related to the Th17 cell phenotype (10) in RRMS patients (11). More recently we found that $CD4^+$ T cells from stable RRMS produced high basal levels of IL-22 compared to healthy donors and CD28 stimulation up-regulated IL-22 gene expression. By using specific inhibitory drugs, we also observed that the up-regulation of IL-22 was dependent on CD28-mediated PI3K activation. Our data identify CD28 as a receptor molecule that may contribute to the inflammatory response in MS by amplifying IL-22 production by Th17 and/or Th22 cells thus favouring the disease progression.

Metodologie:

- Isolation of T lymphocytes from peripheral blood of RRMS patients or HD and stimulation: gradient centrifugation, magnetic separation isolation and cell stimulation with agonistic anti-CD28.2 antibodies (Abs) or recombinant human B7.1/CD80 Fc chimera.
- **Analysis of IL-22 production and IL-22-producing T cell amplification:** the gene expression of IL-22 and related pro-inflammatory cytokines, such as IL-6 and IL-17A, will be analysed by real-time PCR (qPCR) and secretion in culture supernatant will be measured by ELISA.
- **Identification of the specific T helper (Th) cell subset producing IL-22**: staining of specific surface and intracellular markers followed by multicolour flow cytometry technique.
- **Characterization of transcription factors regulating CD28-mediated transcription and secretion of IL-22**: chromatin immunoprecipitation (ChIP) experiments followed by quantitative real-time PCR with SYBR Green Supermix will be performed for the human IL-22 promoter and the specific enrichment will be calculated.
- Functional relevance of CD28-mediated IL-22 production and IL-22-producing cell amplification in astrocyte and oligodendrocyte functions: 1. proliferation and cell death/apoptosis in both astrocytes and oligodendrocytes will be analysed by flow cytometry by carboxyfluorescein succinimidyl ester or 7-ADD, or Annexin V staining; 2. production of metalloproteases MMP2 and MMP9 will be evaluated by measuring secretion of MMP2 and MMP9 in culture medium by ELISA.
- Statistical analysis: Parametrical statistical analysis (mean and standard deviation) will be performed to evaluate differences between continuous variables through Prism 5.0 (GraphPad Software, San Diego, CA) using standard unpaired *t*-test. For multiple group comparisons, significant differences will be calculated using the nonparametric Mann–Whitney *U* test, and linear regression analysis will be performed using the Pearson chi-squared test. For all tests, p values < 0.05 will be considered significant.

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