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| Sommario | T helper (Th) 17 cells are a subpopulation of CD4 T lymphocytes characterized by the expression of interleukin (IL)-17 and the transcription factor retinoid acid receptor-related orphan receptor (ROR)gt. Pathogenic role of human Th17 cells has been demonstrated in several autoimmune diseases, including multiple sclerosis (MS), where they promote inflammatory processes. However, the mechanisms leading to the pathogenicity of Th17 cells are largely unknown. The main objective of this thesis was to identify new mechanisms and molecules inducing inflammatory functions of human Th17 cells that could be potentially involved in the pathogenic processes of MS. We addressed this aim by using two distinct approaches: 1) analysis of general inflammatory features of Th17 cells acquired during Th17 cell differentiation; 2) analysis of intrinsic features of Th17 cells derived from MS patients. In order to investigate potential mechanisms responsible for pathogenic functions of human Th17 cells, we dissected their differentiation process by performing a transcriptome analysis of cells at 48 hours and 5 days of differentiation. We uncovered three time-regulated modules: early modulation, involving exclusively ‘signalling pathways’ genes; late modulation, characterized by genes involved in response to infections; persistent modulation, involving |

effector immune functions. To assign them an inflammatory or regulatory potential, we compared Th17 cells differentiated in presence or absence of IL-1b, respectively. We named inflammatory Th17 condition the polarizing milieu containing IL-1b, which is crucially involved in the pathology of Th17-related diseases. In contrast, Th17 regulatory condition refers to the anti-inflammatory IL-10 cytokine produced in Th17 condition lacking IL-1b. We found that most part of the inflammatory genes belong to the persistent or late module, indicating the crucial role of these genes in the late phases of differentiation. Thus, we elucidated the global molecular signature that characterizes the acquisition of the inflammatory profile by human Th17 cells, by analysing all genes differentially expressed in regulatory versus inflammatory Th17 conditions. Among inflammatory genes, we identified those sharing pathogenic functions with murine Th17 cells, including IL17A, IFNG, TBX21, EBI3, IRF8, TNFRSF9, TNFRSF14, CCL5, CD40LG, BATF and TNF. In addition, our analysis allowed the identification of novel effector molecules, including interferon (IFN)K, lymphotoxin (LT)-a, IL1A, platelet derived growth factor (PDGF)-A, and transcriptional regulators, such as transcriptional regulators homeodomain-only protein homeobox (HOP)X, SRY (sex determining region Y)-box 2 (SOX2), expression of which was independently validated. In order to unveil the mechanisms underlying the acquisition of the human Th17 signature, we investigated the potential transcription factors involved in this process. In this context, we analysed the role of RORgt, known master regulator of Th17 cells, and of the two novel transcriptional regulators HOPX and SOX2, by performing RNA-interference experiments. We found that HOPX regulates IL-17A and IFN-g, while SOX2 regulates PDGF-A and IFN-g. As expected, RORgt regulates expression of IL-17A, IL-17F, but also IFN-g, PDGF-A, and IL1A, not previously described. These results, together with the reduced expression of both HOPX and SOX2 in RORC-interfered cells, suggest that HOPX and SOX2 are two transcriptional regulators acting downstream of RORgt signalling in human Th17 cells. In the second approach, we studied the pathogenic features intrinsically associated to Th17 cells using Th17 cells obtained from MS patients. In particular, we compared in-vitro differentiated Th17 cells of MS patients and healthy donors (HD) and we systematically analysed typical features of Th17 cells, including receptors, transcription factors and soluble factors by flow cytometry, ELISA and Luminex assays. We also included in this study the expression analysis of PDGF and LT-a, two novel effector molecules that we found associated to inflammatory Th17 cells in the previous approach. Results from these analyses unveiled the increased expression of pro-inflammatory proteins IL-21, IL-2, and IL-1 receptor1 (IL-1R1) in Th17 cells derived from MS patients compared to those from HD. Moreover, we found that Th17 cells derived from MS patients express higher levels of LT-a compared to those from HD, indicating that the pathogenic signature that we previously identified contains intrinsic inflammatory features of Th17 cells derived from patients affected by Th17-related diseases. In conclusion, the main results of the project were: 1) the identification of the inflammatory signature of human Th17 cells, that includes novel Th17 genes, such as IFNK, IL1A, PDGF-A, and LT-a, and novel transcriptional regulators HOPX and SOX2; 2) the identification of the intrinsic Th17 features specifically overexpressed in Th17 cells of MS patients, that includes IL-21, IL-2, IL-1R1, and LT-a. Importantly, these factors could become new biomarkers or new therapeutic targets in Th17-related

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