ALTERATIONS IN THE PHENOTYPIC LANDSCAPE AND SPECIFICITY OF CD4+ T CELLS IN CCP+ AT-RISK SUBJECTS BEFORE THE ONSET OF RHEUMATOID ARTHRITIS

Benaroya Research Institute, Translational Research, Seattle, United States of America;
Benaroya Research Institute, Bioinformatics, Seattle, United States of America;
University of Colorado School of Medicine, Division of Rheumatology, Aurora, United States of America;
Janssen Pharmaceuticals, Spring House, United States of America

Background: The “Targeting Immune Responses for Prevention of RA” (TIP-RA) collaboration studies individuals at high risk for developing rheumatoid arthritis (RA) because of serum anti-citrullinated protein antibody (ACPA) positivity in absence of arthritis at baseline, and is focused on defining how they transition from at-risk to classifiable disease. One potential mechanism is the expansion of antigen specific T cells that recognize self-antigens and acquisition of disease associated T cell phenotypes. ACPA emerge years prior to clinically apparent disease and subsequently increase in their titer and breadth of specificity. However, few studies have characterized T cells during this transition.

Objectives: To identify features associated with progression to RA by examining the specificity and surface phenotype of CD4+ T cells in individuals from the TIP-RA cohort by HLA class II tetramer staining and bulk flow cytometry.

Methods: Tetramer staining and flow cytometry were performed on peripheral blood samples from a baseline visit from CCP3+ controls (n=34), CCP3+ at-risk (n=26), CCP3+ positive individuals who transitioned in the near-term to RA (called “RA converters”, n=4), and seropositive early-RA (n=21). Our staining panel allowed us to measure the frequencies of T cells specific for citrullinated alpha-enolase, aggrecan, cartilage intermediate layer protein (CILP), fibrinogen and vimentin. We then applied both supervised phenotyping and a cluster-based computational approach to compare the phenotypic landscape and specificity of antigen specific and total CD4+ T cells in each cohort.

Results: We observed higher overall frequencies of T cells that recognize citrullinated epitopes in CCP3+ at-risk subjects than CCP- controls (p<0.05). Among the individual specificities, elevated frequencies prior to disease onset were most prominent for CILP specific T cells. Supervised phenotypic analysis revealed an increase in CCR4+ CD4+ T cells in CCP3+ at risk subjects (p<0.001) and a corresponding decrease in CXCR3+ CD4+ T cells that was most pronounced in RA converters and seropositive early-RA (p<0.05). Cluster-based phenotypic analysis defined ten distinct phenotypic states present within all subjects. Each of these ten immunotypes contained T cells that recognize citrullinated epitopes. However, the predominant immunotype varied for different antigens. During progression, the frequencies of Ag specific T cells diminished when onset was imminent, but rebounded shortly after diagnosis. Concomitantly, Ag specific T cells with memory phenotypes were diminished, but subsequently reverted to TSCM, Th1, and Th1-17 like phenotypes.

Conclusion: Our data show that disease associated changes in the antigen specificity of CD4+ T cells is present in CCP3+ at-risk subjects. Furthermore, the number of antigen specific T cells and their phenotype are perturbed before the onset of symptoms and development of clinical RA. These findings support a continuum of immunologic changes that underlie risk and drive disease, motivating new approaches for early intervention.

Acknowledgements: We gratefully acknowledge the Targeting Immune Responses for Prevention of Rheumatoid Arthritis (TIP-RA) for designing and executing this collaborative study.

CD4+ T FOLLICULAR AND PERIPHERAL HELPER CELL DIFFERENTIATION IN SYSTEMIC LUPUS ERYTHEMATOSUS

A. Pappalardo, E. Wojciechowski, I. Ondrózola, J. Douchet, N. Merillon, A. Boizard-Moracchini, P. Duffau, E. Lazoraz, M. A. Doucey, L. Mbow, C. Riches, P. Bianco, F. University, CNRS-UMR164, ImmunoConCet, Bordeaux, France; 2Bordeaux University Hospital, Immunology and Immunogenetic Department, Bordeaux, France

Background: Neutrophils have been described as potent antigen-presenting cells that can activate T cells through MHC/TCR interaction and costimulatory molecules in tumor immunity. However, little is known about the direct interaction between neutrophils and CD4 T cells with respect to systemic lupus erythematosus (SLE). We have previously shown that OX40L expression is induced by monocytes from SLE patients promote the differentiation of naive and memory cells into IL21 secreting T cells that are able to help B cells.

Objectives: In this study, we investigate OX40L expression on neutrophils from SLE patients and contribution of these OX40L+ neutrophils in SLE pathogenesis to modulation of the B cell helper role of CD4 T cells.

Methods: Surface expression of co-stimulatory molecules (OX40L, ICOSL, GITRL, 4-1BBL) on neutrophils from SLE patients and healthy donors (HD) was measured by flow cytometry (FC). Neutrophils from HD were stimulated with TLR7 or TLR8 agonists and IFNα after 5 hours of culture. OX40L expression was measured by FC and western blotting. CD4 T cells were cultured with the stimulated neutrophils for 3 days. At the end of the co-culture, percentages of...