THU0033

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

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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 multi-parameter 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 are 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 classified RA. These findings support a continuum of immunologic changes that underlie risk and drive disease, motivating new approaches for early intervention.

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THU0034

AUTOREACTIVE B CELLS ESCAPE PERIPHERAL CHECKPOINT IN ANCA-ASSOCIATED VASCULITIS AND SJOEGREN'S SYNDROME

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Background: B cells play a central role in many autoimmune diseases (AIDs) including ANCA-associated vasculitis (AAV) and primary Sjögren's syndrome (pSS). Most of the research that has been conducted on AID has focused on the production, secretion, and pathogenicity of auto-antibodies, but little is known on the characteristics of autoreactive B cells in humans.

Objectives: This study aims at characterizing circulating autoantigen (PR3 ou SSA)-specific B-cells in patients with AAV and pSS compared to healthy subjects to better understand their role in the **natural and pathological autoimmunity** and define the mechanisms leading to the breakdown of self-tolerance in patients with AID.

Methods: First, we developed a new flow-cytometry method to detect circulating auto-reactive B cell based on the specificity of their B-cell receptor (BCR). To study surface phenotype of specific B cells by flow cytometry, blood samples were collected from patient with PR3-ANCA AAV, pSS and from healthy subjects. Functional analysis of antigen-specific B cells was also elicited by *in vitro* analysis of their capacity to secrete immunoglobulins against SSA or PR3 antigens by ELISPOT

Results: Phenotype analysis showed that antigen-specific B cells in patients have a memory phenotype compared with healthy controls (**5 to 9% are IgG-expressing memory B cells**). It suggests that in AID, these auto-reactive cells are able to differentiate into IgG isotype-switched cells and escape peripheral tolerance checkpoint but not in healthy subjects. Interestingly, Natural auto-reactive B cells are able to secrete only IgM isotype autoantibodies upon *in vitro* stimulation but not IgG class switched antibodies. In order to better understand what differentiates auto-reactive B cells and the mechanisms leading to pathological autoimmunity, a **genomic analysis** of the antibody repertoire as well as a **transcriptional profiling** of these cells by single-cell RNA seq is ongoing to understand further the differences of these autoreactive B cells between healthy subjects and patients with AIDs.

Conclusion: We developed a technology to identify and isolate antigen-specific B cells from the peripheral blood of patients with AID. Our results suggest that autoreactive B cells escape peripheral tolerance checkpoint and are able to differentiate into IgG isotype-switched cells in patients with AIDs but not in healthy subjects.

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THU0035

OX40L EXPRESSING NEUTROPHILS INDUCE CD4 T FOLLICULAR AND PERIPHERAL HELPER CELL DIFFERENTIATION IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Neutrophils have been described as potent antigen-presenting cells able to activate T cells by 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 expressed by monocytes from SLE patients promote the differentiation of naïve and memory cells into IL21 secreting T cells that are able to help B cells^{1,2}.

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