

Poster Tours

Adaptive immunity (T cells and B cells) in rheumatic diseases

POS0001

PHENOTYPE AND FUNCTIONAL CHARACTERISTICS OF ANTIGEN-SPECIFIC, AUTO-REACTIVE B CELL RESPONSES REVEAL DIFFERENTIAL IMMUNOLOGICAL ACTIVITY IN PATIENTS WITH SYSTEMIC SCLEROSIS

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Background: Systemic Sclerosis (SSc) is a systemic autoimmune disease that carries the highest mortality burden among the rheumatic diseases. Disease risk and course are difficult to predict in individual patients, and anti-inflammatory and B-cell depleting therapies show varying results. >95% of SSc patients harbor autoantibodies. Among those, anti-topoisomerase antibodies (ATA) and anti-centromere antibodies (ACA) are most prevalent, mutually exclusive in individual patients and associate with distinct disease phenotypes. Despite these associations, the clinical value of both ATA and ACA for patient stratification within these phenotypes is limited. Here, we hypothesized that phenotypic and functional characteristics of the underlying autoreactive B cell responses could allow insights in differential 'immunological disease activity' in individual patients, thereby providing indications as to potential drivers of these responses as well as granularity as to which patients may benefit from targeted interventions.

Objectives: To assess phenotypic and functional characteristics of anti-topoisomerase and anticentromere specific B cell responses in individual patients with SSc.

Methods: Peripheral blood mononuclear cells (PBMC) from ATA- and ACA-positive SSc patients were cultured without stimulation or in the presence of CD40L-expressing fibroblasts, IL-21 and BAFF. Following culture, ATA- and ACA-IgG and -IgA were measured in culture supernatants by ELISA. In addition, PBMC were depleted of circulating plasmablasts by fluorescence activated cell sorting (FACS), and isolated plasmablasts were cultured separately. Furthermore, the presence of antigen-specific plasmablasts was confirmed by ELISPOT. Finally, the degree of spontaneous ATA secretion was correlated to the presence or absence of interstitial lung disease (ILD; based on high-resolution computed tomography). Healthy donors and patients with rheumatoid arthritis served as controls.

Results: We observed that individual ATA- and ACA-positive SSc patients harbored circulating B cells that secrete either ATA-IgG or ACA-IgG upon stimulation, depending on their serotype. In addition, we noted spontaneous secretion of ATA-IgG and, more remarkably, extensive secretion of ATA-IgA in ATA-positive patients. This degree of spontaneous, antigen-specific IgA secretion was specific for the ATA response, while spontaneous ACA-IgA secretion was undetectable in patients harboring ACA. FACS experiments and ELISPOT showed that the spontaneous ATA-IgA and -IgG secretion was attributable to circulating plasmablasts. Of note, the degree of spontaneous ATA-IgG secretion was remarkably higher in patients with ILD than in those without.

Conclusion: Our findings demonstrate that individual ATA-positive SSc patients harbor activated ATA-IgG and ATA-IgA B cell responses, as indicated by the spontaneous secretion of both ATA isotypes by circulating plasmablasts. Importantly, by taking the presence of plasmablasts as a proxy for recent B cell activation, our data suggest a link between the activity of the antigen-specific B cell response and the presence of ILD. In contrast, the ACA B cell response was far less active and lacked the active IgA component, which suggests a difference in the triggers driving these autoreactive B cell responses in patients. In fact, the remarkable ATA-IgA secretion points towards a potential mucosal trigger of the ATA response, which may be continuously active in individual patients.

Disclosure of Interests: None declared.

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POS0002

PI16 REPRESSES FOXP3 EXPRESSION IN T REGULATORY CELLS AND EXACERBATES AUTOIMMUNE ARTHRITIS VIA INHIBITING THE K48-LINKED POLYUBIQUITIN DEGRADATION OF BMI-1

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Background: Regulatory T cells (Tregs) play an essential role in maintaining self-tolerance and immune homeostasis. Abnormalities in the quantity or function of Treg cells are believed in RA patients, contributing to the inability to suppress autoimmunity and proinflammatory cytokines. Forkhead box P3 (Foxp3) is a crucial transcription factor for the development and differentiation of Tregs. How Tregs lose Foxp3 expression under inflammatory milieu remains largely unknown. Peptidase inhibitor 16 (PI16) is a member of the CAP (Cysteine-rich secretory proteins, Antigen 5, and Pathogenesis-related 1) protein family and its function are largely poor understood. In a genome-wide expression profiling study for identifying human Foxp3 target genes revealed PI16 was expressed on the cell surface of >80% of resting human CD25⁺ Foxp3⁺ Tregs. In the inflamed joint of juvenile idiopathic arthritis revealed a low number of PI16⁺ Tregs but high number of Th17 cells. However, little is known the function role of PI16 on Tregs or on RA development.

Objectives: To investigate the role of peptidase inhibitor 16 (PI16) on the key T regulatory (Tregs) cells transcription factor Foxp3 expression and on the development of autoimmune arthritis.

Methods: The expression of PI16 in blood, synovial fluid, inflamed joints were examined in Rheumatoid arthritis (RA) patients and in arthritic mice. Arthritis symptom, histological features and Foxp3 expression in PI16 transgenic (PI16^{Tg}) arthritic mice were examined. Posttranslational mechanisms on PI16-mediated Foxp3 expression were analyzed. The specific role PI16 on Foxp3 expression was validated in conditional knockout (KO) mice.

Results: The expression of PI16 was significantly increased in PBMC, serum, synovial tissue from RA patients or arthritic mice compared with controls. PI16^{Tg} arthritic mice exhibited obvious inflammation, synovial hyperplasia and articular cartilage destruction in the joints compared with those in wild-type mice (WT) arthritic mice.

Foxp3 is downregulated in splenic T cells and synovial tissue from PI16^{Tg} arthritic mice. Naïve T cells derived from PI16^{Tg} arthritic mice showed the decreased capacity to differentiate into Tregs. Polycomb-group (PcG) proteins complex molecule of Bmi-1 was significant increase in Tregs and joint tissue from PI16^{Tg} arthritic mice. A direct interaction between 1-95AA domains of PI16 and 169 and 436 domains of Bmi-1 in Tregs promoter was observed. The binding of PI16 with Bmi-1 in the Foxp3 promoter inhibit the K48-linked polyubiquitin degradation of Bmi-1 at lysine site 72 and 153 region, which prompts the repressive histone modification of H3K27me3 and H2AK119ub, and inhibits the active histone modification of H3K4me3. Furthermore, conditional knockout of PI16 in Tregs retarded Foxp3 loss and blunted disease progression in experimental arthritis.

Conclusion: PI16 represses Foxp3 expression by mediating histone modification via inhibiting K48-linked polyubiquitin degradation of Bmi-1 in Foxp3 promoter, contributing to disease progression in arthritic mice.

Disclosure of Interests: None declared.

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POS0003

RITUXIMAB THERAPY IN SYSTEMIC LUPUS ERYTHEMATOSUS – TRANSIENT EFFECTS ON AGE ASSOCIATED B-CELLS

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Background: Immune system's abnormalities in SLE involve several subsets of the B-cell compartment, including double negative B-cells (DN) and CD11c+CD21- B cells (also referred to as ABC-age associated B cells), which are expanded in the disease. ABC cells are also known to interact with T helper cells, T follicular and peripheral helper cells (1). Rituximab, a chimeric anti-CD20 antibody, depleting B cells, is commonly used off-label as treatment for SLE patients, especially in lupus nephritis. Little is known on the impact of B-cell depletion on such B-cell subsets and on B-T-cell interactions.

Objectives: to investigate the effects of rituximab (RTX) on the frequencies of double negative B-cell subsets and CD11c+CD21- ABC cells and as well as T follicular helper (T_{FH}, CXCR5+ PD-1+) and T peripheral helper (T_{PH}, PD-1^{high}) CD4+ T-cell subsets.

Methods: 15 SLE patients, starting RTX and followed longitudinally up to two years, were analyzed for lymphocyte subsets using multicolor flow cytometry. Cryopreserved PBMC were thawed and stained at the same time together with one buffy coat. Around 1 x 10⁶ PBMC for each panel were labeled and further stained with fluorescent antibodies for B and T-cell markers. For the B-cell panel, PBMC were stained with anti-CD3, CD14, CD16, CD19, IgD, CD27, CD38, CD11c, CD21 and in some samples with anti-CXCR5 antibodies. For the T-cell panel, PBMC were labeled with anti-CD16, CD14, CD19 and CD3, CD4, CD8,