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.

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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,

PD-1, CCR7, CXCR5, CD45RA antibodies. All patients fulfilled the ACR 1982 classification criteria for SLE. Cellular changes were analyzed in the context of clinical information.

Results: In the present cohort, the SLE patients were mainly female (86.6%) and of median age of 36.7 (29.8-49.4) with a disease duration of 6.1 (1.6-11.8) years, and active disease with SLEDAI-2K at baseline 12.0 (8.0-16.0). The frequency of age-associated B cells (ABCs; CD27-IgD-CD11c⁺ CD21⁻) decreased by 13% (p=0.03) in the first two to four months after rituximab start, while globally the DN (IgD-CD27⁺) B cells transiently increased by around 3% (p=0.15) at the first follow-up. This increase could not be attributed to the DN1 (CXCR5+CD11c⁻) or DN2 (CXCR5-CD11c⁺) subsets but to the CD11c-CXCR5- DN (DN3) B cells (increase= 6.7%, p=0.03). In parallel, T effector cells (CCR7- CD45RA⁺) and TEMRA (CD45RA⁺ CCR7⁻) frequencies increased after first follow up in both CD4⁺ and CD8⁺ T cells. The frequency of T_{HH} (CXCR5+ PD-1⁺) cells did not change after rituximab, however a decrease of PD-1^{high} CD4⁺ cells was observed in most patients, although not significant, after 2-4 month of treatment. In most patients the frequency of PD-1^{high} CD4⁺ cells either reduce or stay the same after RTX treatment (reduction= 0.53, p=0.28). After 11-15 months of RTX treatment the frequency of PD-1^{high} CD4⁺ T cell reduces by a -0.5% in comparison to 2-4 months (p=0.039). The SLEDAI at baseline did not correlate with the frequency of PD-1^{high} CD4⁺ T cells (r=0.03, p=0.9).

Conclusion: the importance of T cell - B cell interactions in SLE pathogenesis was recently strengthened by the identification of the lymphocyte subsets T_{HH}/T_{PH} and ABCs respectively. Here, in the context of rituximab treated SLE, we could detect a reduction in the frequencies of both ABCs and PD-1^{high} T cells after treatment with rituximab, while the DN3 and effector memory T cells frequencies increased. Our data suggests that anti-CD20 mediated B-cell depletion affects both B-cell and T-cell subsets frequencies, and that monitoring these specific cell subsets may be clinically relevant.

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POS0004

T-CELL REPERTOIRE OF SYNOVIAL FLUID IN SPONDYLOARTHROPATHIES EXHIBITS HALLMARKS OF HLA-DEPENDENT CLONAL EXPANSIONS AND REMAINS STABLE OVER 1.5 YEARS

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Background: Different studies show involvement of T-cells in pathogenesis of spondyloarthropathies (SpAs) - a group of rheumatic diseases strongly associated with presence of several MHC-I alleles (HLA-B*27, -B*38, -B*39, etc). Recently we and others identified a specific T-cell receptor motif in blood and synovial fluid of HLA-B*27+ AS patients that reinforces the "arthritogenic peptide" hypothesis of AS pathogenesis [1-2]. However, common characteristics of clonal T-cell repertoire of synovial fluid in SpAs remain poorly investigated.

Objectives: We aimed to investigate synovial fluid T-cell repertoires of SpA patients with different HLA-genotypes and stability of the clonal composition in recurring flares of the disease.

Methods: Mononuclear cells were isolated from paired peripheral blood (PB) and synovial fluid (SF) samples of SpA patients (ankylosing spondylitis and psoriatic arthritis, n=27). For 3 patients additional SF samples were collected during relapse of the synovitis (after 9-15 months). CD4 and CD8 T-cells were isolated by immunomagnetic separation. Deep sequencing of UMI-tagged TCR beta cDNA libraries was used to accurately reconstruct clonal T-cell repertoires. HLA class I and II were typed for each donor using an in-house NGS-based system.

Results: We observed restricted T-cell clonal composition in synovial fluid: on average only 6% of PB T-cell clonotypes were detected in SF of the same donor. T-cell repertoires of both CD4 and CD8 SF subsets compared to PB were highly oligoclonal (index Gini PB vs SF: CD4 0.36±0.10 vs 0.68±0.08, CD8 0.57±0.17 vs 0.81±0.12) in all patients. Number of identical amino acid CDR3 sequences between two repertoires correlated with the number of identical HLA-alleles for the donors. This trend was exhibited more strikingly in SF compared to PB,

suggesting that common antigens may play a role in accumulation of identical T-cell clonotypes in the inflamed joint. Using several bioinformatic approaches we identified groups of highly similar SF clonotypes linked to HLA-B*27 and/or HLA-B*38 genotype.

Total SF repertoires of relapsing synovitis of the same donor showed huge clonal overlap, and the most frequent clonotypes remained almost unchanged (Morisita's overlap index for total SF repertoires 0.69±0.26; for top 1000 clonotypes 0.79±0.19, n=3).

Conclusion: We report HLA-dependent sharing of identical and similar T-cell clonotypes in SF of patients with ankylosing spondylitis and psoriatic arthritis and high stability of SF repertoire during several flares that support antigen-driven accumulation of T-cells in the site of inflammation.

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POS0005

THE EFFECT OF LKB1 INHIBITION IN RA PATHOGENESIS

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Background: Liver kinase B1 (LKB1) is known as a tumor suppressor gene and also inhibits reactive oxygen species (ROS) levels. Intracellular ROS are catalyzed by the enzyme complex nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX). We previously reported that NOX4 induced the migration and invasion of fibroblast-like synoviocytes (FLS) in rheumatoid arthritis (RA). Although LKB1 is expected to alleviate synovial inflammation through ROS regulation, the role of LKB1 in RA has not been examined.

Objectives: To explore whether LKB1 affects RA inflammation, we transfected LKB1 siRNA and analyzed related gene expressions in RA FLS.

Methods: Synovial tissues were obtained from RA patients who were undergoing synovectomy or joint replacement. The isolated cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 mg/ml streptomycin and maintained in a 5% CO₂ incubator at 37 °C. FLS were used for experiments after four to six passages. Cells were transfected with lipofectamine transfection reagent and LKB1 siRNA duplex targeting constructs. After incubation for 24h, downregulation of target expression was evaluated by real-time PCR and western blot analysis.

Results: RA FLS was transfected with LKB1 siRNA and 90% of LKB1 mRNA expression was decreased. LKB1 knock-down also caused the decreased expression of mechanistic target of rapamycin (mTOR; 0.38 fold) and serine/threonine kinase (AKT) 2 (0.40 fold), which are downstream targets of LKB1. NOX4 was significantly increased (4.94 fold) by LKB1 inhibition. On the other side, the down regulated NOX4 induced significantly elevated LKB1 mRNA expression in RA FLS. When the expressions of proinflammatory cytokines were examined, IL-1β, IL-6, TNF-α were highly increased by LKB1 deficiency. FLS migration-related chemokines, IL-8 and MMP-3 were also enhanced compared to control.

Conclusion: There was a negative correlation between NOX4 and LKB1 in RA FLS. As LKB1 deficiency induced the expression of proinflammatory cytokines and migration related chemokines, LKB1 could play a critical role in RA pathogenesis.

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POS0006

NX-5948, A SELECTIVE DEGRADER OF BTK, SIGNIFICANTLY REDUCES INFLAMMATION IN A MODEL OF AUTOIMMUNE DISEASE

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