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

POS1008 INVESTIGATING ANTIGEN SPECIFIC T CELLS AND THEIR CITRULLINATED PROTEIN TARGETS IN SYNOVIAL TISSUE

Keywords: Adaptive immunity, Rheumatoid arthritis, Synovium

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Background: Rheumatoid arthritis (RA) is a T cell mediated autoimmune disease in which citrullinated self-antigens are recognized by anti-citrullinated protein antibodies (ACPA) and T cells. To date, the majority of T cell studies have been performed using peripheral blood and have focused on well-documented ACPA targets. Studies of disease-affected tissue are needed to confirm and extend observations that have been made through study of peripheral blood T cells.
Objectives: T cell subsets and targets that have not been observed in peripheral blood are likely to comprise an important (but as yet, understudied) component of the antigen specific responses that underlie RA. We sought to generate novel insights about T cell phenotypes and antigenic targets by performing multicolor flow cytometry analysis and HLA peptidomics studies of synovial tissue samples from subjects with RA.
Methods: Synovial tissue was obtained from 7 subjects with seropositive RA, 4 subjects with seronegative RA, and 8 subjects with osteoarthritis, all of whom had undergone arthroplasty procedures. Synovial cell suspensions were obtained from tissue through mincing, digestion in collagenase I, and filtration. These were subjected to multicolor flow cytometry analysis to gain insights about the lymphocyte and non-lymphocyte cell subsets present. After confirming leukocyte antigen (HLA) protein expression by flow cytometry, additional tissue was solubilized in lysis buffer and HLA class I and HLA-DR complexes were captured (separately) on affinity columns. HLA-bound peptides were eluted, concentrated, and peptide spectra were identified by LC-MS/MS analysis. The resulting datasets were assigned sequences by searching against a human protein database. Mass shifts associated with each assigned sequence were utilized to identify post-translational modifications – most notably citrullination of native arginine residues. Comprehensive libraries of the HLA-Class I- and HLA-DR-bound peptides from each individual were imported into a custom database, which was then used to catalogue the most prevalent self-proteins for each patient type. T cell assays were then performed to demonstrate the immunogenicity of novel targets and probe T cell antigen specificity in tissue.
Results: Flow cytometry analysis of synovial tissue derived cells demonstrated that fibroblasts (including fibroblast-like synoviocytes), monocytes, and B cells were all present in tissue. In particular, fibroblasts and monocytes showed evidence of inflammation, including upregulated levels of HLA-DR expression. Similar numbers of CD4+ and CD8+ T cells were present in tissue, with phenotypes that included various memory subsets but essentially no naïve cells. In comparison with peripheral blood, T cells from synovial tissue showed evidence of recent activation, expressing higher levels of CD95, CD71, PD-1, ICOS, and CD69. The most prevalent self-proteins in the HLA-DR-bound peptidome from the synovial tissue of RA subjects included expected targets such as vimentin, alpha-enolase, fibrinogen, collagen, histones, and BIP but also contained novel targets such as fibronectin, gelsolin, and proteoglycan 4. Prevalent self-proteins in the HLA-Class I-bound peptidome included well-studied CD4+ T cell targets such as vimentin, alpha-enolase, and collagen but also contained more novel targets such as caspase-14, stromelysin-1, and filamin-A. T cell assays supported the immunogenicity of expected targets and the new candidate antigen gelsolin. In particular, a comparatively robust population of aggrecan specific T cells was present in tissue.
Conclusion: Our findings demonstrate that flow cytometry and HLA peptidomic analysis of synovial tissue can provide novel insights about the phenotype and antigen specificity of T cells in RA. Further characterization of T cell response in tissue, including those that recognize novel antigens, has the potential to provide important new insights about the character of antigen specific T cell responses that promote the development of RA.
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Innate immunity in rheumatic diseases

POS1009 RADIOGRAPHIC AIRWAY ABNORMALITIES IN UNTREATED EARLY RHEUMATOID ARTHRITIS ARE ASSOCIATED WITH PERIPHERAL NEUTROPHIL ACTIVATION

Keywords: Lungs, Innate immunity, Rheumatoid arthritis

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Background: The role of the lung for the initiation and progression of rheumatoid arthritis (RA) is still unclear [1]. Up to 10% of RA patients develop severe treatment resistant lung disease [2]. Understanding early disease mechanisms is of great importance. Neutrophils are key players in RA pathogenesis and are recruited to the lungs early in the disease [3]. In RA, circulating neutrophils display an activated phenotype and neutrophil activation markers predict joint destruction and development of extra-articular nodules [4]. Neutrophil activation status has not been studied in relation to pulmonary abnormalities (PA) in early untreated RA (ueRA).
Objectives: To determine whether there is an association between peripheral neutrophil phenotypes and presence of PA on chest high-resolution computed tomography (HRCT) in ueRA.
Methods: Clinical data and blood were collected, and HRCT performed at diagnosis on 30 consecutive anti-citrullinated protein antibody (ACPA) and/or rheumatoid factor (RF) positive ueRA patients. HRCTs were evaluated for the presence and extent of RA-associated parenchymal, airway and/or pleural abnormalities. Expression of phenotype markers on neutrophils separated by density was determined by flow cytometry. Levels of calprotectin, ACPA and RF were measured using immunoassays. An initial principal component analysis was used to visualize the relationships of the multidimensional data followed by univariate analysis of the strongest associations.
Results: The median patient-reported symptom duration was 6 months, 20 % of the patients were current smokers and the mean disease activity was moderate in this seropositive ueRA cohort. The frequency of having any PA detected by HRCT was 60 %. Airway abnormalities were present in 50%, nodules in 43 % and interstitial lung abnormalities (ILA) in 10 %. Unsupervised multivariate factor analysis showed clustering of "any PA" with neutrophil activation, parameters of inflammation and RF titres (Figure 1A). Univariate analysis confirmed a significantly increased CD11b and decreased CD62L expression on neutrophils indicating activation in patients with PA as compared to no PA. Titres of RF, but not ACPA, correlated with expression of the neutrophil activation marker CD11b. A stratified analysis demonstrated that airway involvement was the PA subtype with the strongest association with neutrophil activation (CD11b 1.3-fold, p=0.014 and CD62L 0.6-fold, p=0.003 in patients with airway abnormalities as compared to no PA) and with RF IgM titres (8.8-fold, p=0.0002) (Figure B-E).
Conclusion: We report a significant association between radiographic airway findings and activation of circulating neutrophils in early RA supporting a role of innate immunity and the lung in disease onset. Our results also indicate different contributions of RF and ACPA in the RA pathogenesis. Parts of this abstract was presented 15 September 2022 on a national rheumatology meeting in Gothenburg, Sweden.

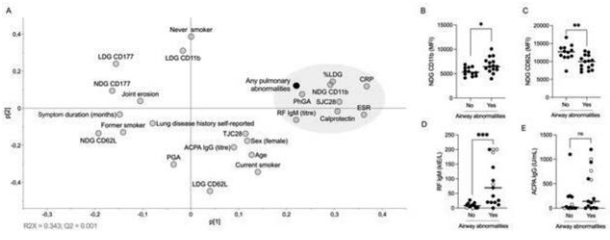

Figure 1. Principal component analysis showing the relationship between the presence any pulmonary abnormalities by HRCT, neutrophil phenotypes, disease activity measures and demographic data in ueRA patients (n=30) (A). Univariate analysis of CD11b (B) and CD62L (C) expression, RF (D) and ACPA titres (E) in patients with airway abnormalities vs no PA. Bars show median. NDG=normal density granulocytes, LDG=low density granulocytes. Smokers open circles.

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POS1010 MACROPHAGE EXTRACELLULAR TRAPS PROMOTE FIBROBLAST-LIKE SYNOVIOCYTES PROLIFERATION AND PROINFLAMMATORY CYTOKINE IN RHEUMATOID ARTHRITIS

Keywords: Innate immunity, Synovium, Rheumatoid arthritis

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Background: Macrophage Extracellular Traps (METs) play an important role in the promotion of tissue injury, inflammation progression and autoimmune diseases.
Objectives: This study aims to investigate the effects of METs on the proliferation and expression of proinflammatory cytokines in fibroblast-like synoviocytes (FLS) in patients with rheumatoid arthritis (RA).
Methods: Synovial tissues of RA patients and traumatic controls undergoing joint replacement in our hospital were collected, and FLS of RA patients were cultured in vitro. Peripheral blood mononuclear cells (PBMC) were isolated from RA patients and differentiated into macrophages by M-CSF. The macrophages were co-cultured with serum of RA patients and healthy controls. SYTOX green, which stained the DNA released from cells and was a common method for detecting ETs, was added to observe the formation of METs. The expressions of CD68 and CitH3 in synovium were detected by immunofluorescence. METs were isolated and purified from THP-1 derived macrophages and co-incubated with FLS. The RNA expressions of proinflammatory cytokines TNF-a and IL-1β of RA-FLSs were detected by qPCR. The proliferation ability of RA-FLSs was detected by CCK-8 assay.
Results: We found the enrichment of macrophages labeled with CD68 and CitH3 in RA synovial tissues but not in traumatic controls. Immunofluorescence co-localization assay displayed that most CitH3 were distributed around CD68, suggesting that macrophages may be the main source of ETs (Figure 1A). PBMC induced macrophages co-incubated with serum from RA patients showed the formation of strip-like METs, while no obvious METs were observed with serum from healthy controls (Figure 1B). Purified METs were isolated and co-incubated with RA-FLSs for 48h. TNF-a and IL-1β were significantly overexpressed, and the proliferation of RA-FLSs was promoted (Figure 1C-D).
Conclusion: METs were detected in RA synovium but not in traumatic controls. The autoantibodies or inflammatory cytokines presented in the serum or RA patients may be associated with the increased METs formation in RA. In vitro experiments, METs could promote the proliferation and proinflammatory cytokines expression of RA-FLSs, suggesting that clearing or blocking the formation of METs may be a new therapeutic target for RA.

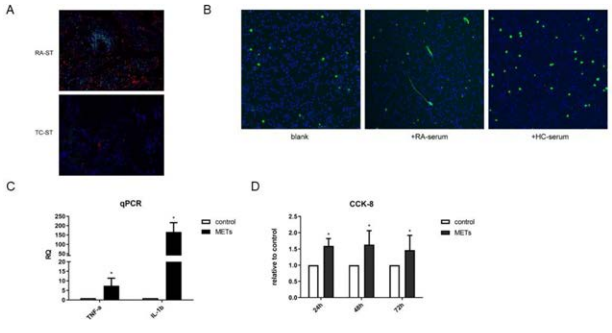

Figure 1. A, Immunofluorescence of synovium in RA and traumatic controls. Immunofluorescence showed the areas stained with anti-CD68 (red), anti-CitH3 (green) and DAPI (blue). RA: rheumatoid arthritis, ST: synovial tissue, TC: traumatic controls. B Formation of METs in human monocyte-derived macrophages with serum SYTOX green showed the formation of METs in macrophage with RA-serum. SYTOX green (green) and Hoechst 33342 (blue). C-D The promotion of METs in biologic behaviors of RA-FLSs. METs could promote the proliferation (C) and proinflammatory cytokines expression (D) of RA-FLSs.

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POS1011 DIFFERENT SYNOVIAL MACROPHAGE AND FIBROBLAST SUBSETS, EXPRESSION PROFILES AND CELL-CELL INTERACTIONS CHARACTERISE SEX DIFFERENCES IN CHRONIC INFLAMMATORY JOINT DISEASES

Keywords: Synovium, Inflammatory arthritides, -omics

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Background: Chronic inflammatory joint diseases differ between males and females in terms of disease manifestation, treatment response and radiographic progression. A molecular basis that could explain these differences has yet to be identified.
Objectives: The study aimed to find differences in synovitis at histology and transcriptome level between males and females with chronic inflammatory joint diseases.
Methods: Synovial tissue was obtained by ultrasound-guided biopsy from inflamed joints from 5 males and 5 age- and disease-matched females. Histological analysis included Krenn score and synovial pathotype. Single-cell RNA-sequencing (scRNA-seq) libraries were prepared with 10X Genomics and sequenced with NovaSeq 6000. We performed additional histological analyses (Krenn score and CD68 staining) on synovial tissue samples obtained from joint replacement surgery in 13 males and 13 age- and synovial pathotype-matched females with rheumatoid arthritis. The following R packages were used for bioinformatic analysis: Cell Ranger, Seurat, Harmony and CellChat. KEGG gene set enrichment analysis was performed with ClusterProfiler.
Results: We included 4 psoriatic arthritis, 2 rheumatoid arthritis, 2 undifferentiated arthritis and 2 peripheral spondyloarthritis patients. Females and males did not significantly differ in baseline characteristics: mean age was 46.2 years in females, 46.8 years in males; 2 knees and 3 wrists were biopsied per sex; mean swollen joint count was 6.8 in females and 8.2 in males. Histological analysis between the sexes showed no significant differences: synovitis was moderate in both sexes (mean Krenn total score 4.0) and pathotypes were balanced in males (1 diffuse-myeloid, 2 lympho-myeloid, 2 pauci-immune) and females (1 diffuse-myeloid, 1 lympho-myeloid, 1 pauci-immune, 2 ungradable). 41,014 cells were integrated for scRNA-seq analysis (female 21,636 and male 19,378 cells). Pseudobulk variance analysis showed a significant correlation of sex with principal component (PC) 3, accounting for 12.6% of the sample variation (Figure 1A). In addition to the sex-specific genes XIST and PRS4Y1, the following genes were main drivers of the PC3 variation: H19, CXCL9 and CXCL10. Diagnosis was not significantly associated with any PC. Comparison of the individual cell cluster proportions showed that LYVE1^{pos} macrophages (MC) were significantly more prevalent in females (4.9% in males versus 13.7% in females, p = 0.03) (Figure 1B). In confirmatory histological analysis, consistent with the known location of LYVE1^{pos} MC in the lining layer, both Krenn lining score (mean (SD) score in males 1.85 (0.69) and 2.08 (0.76) in females) and CD68 lining score (mean (SD) score in males 1.77 (1.17) and 2.08 (0.95) in females) were higher in females but did not reach statistical significance. Differential gene expression analysis showed that mainly in synovial fibroblasts (SF) genes were differentially expressed between males and females (n = 1944). In SF, upregulated genes in males led to a significant enrichment of proinflammatory pathways (TNF pathway, NF-kappa B pathway, IL-17 pathway). In contrast, upregulated genes in females were significantly associated with ECM-receptor interaction, focal adhesion, protein digestion and absorption pathways (Figure 1C). In cell-cell interaction analyses, most outgoing signaling was observed in SF while MC represented most receiving cells; IL6 and CXCL signaling pathways were significantly enriched in males, and COLLAGEN and THY1 signaling pathways in females (Figure 1 D/E).
Conclusion: Our study shows potential important differences in synovitis between males and females with chronic inflammatory joint diseases. In females, synovitis was characterized by abundance of LYVE1^{pos} MC and SF expressing