tenascin specific CD4+T cells, followed by PHA expansion resulted in visible increase in proportion of citrullinated tenascin specific CD4+T cells.

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THU0047

THE SYNOVIUM REQUIRES AN IMMUNOLOGICAL RHEOSTAT THAT DEFINES TWO FUNCTIONALLY DISPARATE PATHOGENIC CD4+HLA-DR+ SUBSETS IN HUMAN ARTHRITIS

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Background: Despite advances in understanding how the adaptive T cell landscape is affected in human arthritis, specific T cell subset knowledge has yet to be utilised in clinical settings. We have previously discovered within active arthritic patients, a circulating pathogenic-like lymphocyte (CPLs; CD4+HLA-DR+) within the T-effector compartment, that is phenotypically similar to their synovial counterparts. CPLs are inflammatory, correlate with disease activity and overlap in synovial TCR repertoire. A similar inflammation-associated T-regulatory (iaTreg; CD4+HLA-DR+) subset, that is activated, poised to migrate to inflamed site and sharing synovial TCR overlap, suggest a common disease ontogeny that may exist between CPLs and iaTregs.

Objectives: Here we seek to determine whether and how the synovial microenvironment plays a role in modulating these two functionally divergent (Teff/Treg compartments) yet pathogenically homologous subsets. This modulation, akin to an immunological rheostat, may be a feature of the disease process.

Methods: We examined CD45+ immune cells from synovial and PBMCs (active JIA, inactive JIA, paediatric healthy) through mass cytometry (CyToF). CD4 T cells were sorted into CPLs, iaTregs, Teff and Treg through FACS Aria II, from active JIA PBMCs, paired JIA SFMCs and healthy paediatric PBMCs and examined through ngRNAseq.

Results: Mass cytometric analysis reveal a significant enrichment of synovium signatures in both circulatory CPLs and iaTregs subsets from active arthritic PBMCs, as compared with the conventional pool of Teff/Tregs. This immunological relationship between CPLs/iaTregs is reaffirmed by comparative differential gene expression (DEG) and phylogenetic tree analysis, which indicated transcriptomic convergence between circulatory pathogenic CPLs/iaTreg subsets and divergence from their respective conventional Teff/Treg pools. Circulatory CPLs/iaTregs exhibit (a) common pathway dysregulation in T cell signalling, (b) restriction in TCR oligoclonality and (c) common transcription factor drivers within the gene regulatory network, suggesting a common pathogenic mechanism acting on these two disparate compartments.

To understand how the microenvironment plays a role in modulating these two subsets, we compared the transcriptome of CPLs/iaTreg and conventional Teff/Treg subsets from (a) healthy PBMCs, (b) JIA PBMCs and (c) paired JIA SFMCs. The convergence between CPLs/iaTreg increases across the spatial/disease continuum, culminating in 7 key common dysregulated pathways within synovium CPLs/iaTregs. Importantly we detected higher clonotypic sharing of TCRs in CPLs/iaTregs across the spatial and disease continuum, suggesting a common precursor driven by antigenic selection.

Conclusion: Our data suggest that CPLs/iaTregs are dichotomic components of a systemic immune rheostat, shape through the synovium environment, modulating autoimmunity in human arthritis. As iaTreg and CPL most likely have the capacity to morph into each other, the molecular crossroads which control this plasticity represent novel therapeutic targets.

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THU0048

THE FUNCTION CHANGES OF SNPS IN THE P2X7 RECEPTOR BY ALA348TO THR, GLU496TO ALA AND ARG307TO GLN IN THP-1 CELLS WITH HIGH URIC ACID

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Background: Previous studies[1] have shown that ATP acts on the sputum receptor P2X ligand-gated ion channel 7 (P2X7R) as a second signal to induce gouty arthritis.

Objectives: In this study, the functional changes of three SNP genotypes, Ala348 to Thr, Glu 496 to Ala and Arg307 to Gln, in P2X7R were analyzed with high uric acid.

Methods: After transfection of HEK-293T cells by lentivirus, observing the uptake ability of HEK-293T cells to ethidium bromide. The effect of three different mutants on the P2X7 receptor was thus observed on the P2X7 channel. In addition, THP-1 cells were also transfected, stable expression of a THP-1 cell line that has been transfected with a wild-type or different mutants and thus established. Then three types were set up separately, and each type was randomized into three groups: MSU(labeled M), MSU+ATP (labeled MA), and unstimulated control group (labeled C). Detection of IL-1 β protein expression level in serum by ELISA and NLRP3, ASC and Caspase-1 mRNA levels in transfected THP-1 cells by qRT-PCR.

Results:

1. These three variants have different effects on the uptake function of ATP-induced ethidium⁺ bromide in transfection of HEK-293T cells by lentivirus. Ala³⁴⁸to Thr increased P2X₇-dependent ethidium⁺ bromide uptake (145% of wild-type P2X₇ response, P<0.001). In contrast, Absent or very reduced P2X₇ function was found in Glu⁴⁹⁶to Ala and Arg³⁰⁷to Gln subjects, appeared to abolish P2X₇-dependent dye uptake (38% and 32% of wild-type P2X₇ responses, P<0.001.), who were compared with wild-type.
2. Compared the IL-1 β levels of the three variants with the wild-type and empty virus in THP-1 cells, the Ala³⁴⁸to Thr mutation significantly up-regulated the serum levels of IL-1 β compared with the wild-type and empty virus in group MA with high uric acid (P=0.0007; P=0.013, respectively). Moreover, similar results have also been shown in IL-1 β mRNA expressions (P=0.0334; P=0.0307, respectively). The Glu⁴⁹⁶to Ala and Arg³⁰⁷to Gln mutations down-regulated the serum levels of IL-1 β compared with the wild-type in group MA (P=0.0189; P=0.0164, respectively).
3. NLRP3 mRNA was significantly increased in the Ala³⁴⁸to Thr mutation compared with the wild-type and empty virus in group MA (p =0.0003; P=0.0001, respectively). However, NLRP3 mRNA was significantly reduced in the Glu⁴⁹⁶to Ala and Arg³⁰⁷to Gln mutations compared with the wild-type in group MA (p =0.0294; P=0.0279, respectively).
4. Wild-type was significantly higher than empty virus in the ASC gene expression in group MA (P=0.0022). Moreover, the Ala³⁴⁸to Thr mutation was higher than empty virus while Arg³⁰⁷to Gln mutation was lower than that in group MA (P=0.0138; P=0.0283, respectively).
5. Unlike NLRP3 gene expression, the data showed that the expression of caspase-1 mRNA in group C, M and MA all with no statistical significance, respectively (P>0.05).

Conclusion: Our data revealed that Ala³⁴⁸to Thr up-regulate the functional status of P2X7R and Glu⁴⁹⁶to Ala and Gln⁴⁶⁰to Arg down-regulate the functional status of P2X7R, which resulted in a significant increase or decrease in IL-1 β and NLRP3 expression levels with high uric acid.

References:

- [1] Tao JH, Zhang Y, Li XP, et al. P2X7R: a potential key regulator of acute gouty arthritis[J]. *Semin Arthritis Rheum*. 2013;43(3):376–380.

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THU0049

DISTINCT EXPRESSION OF COINHIBITORY MOLECULES ON ALVEOLAR T CELLS IN PATIENTS WITH RHEUMATOID ARTHRITIS- AND IDIOPATHIC INFLAMMATORY MYOPATHIES-ASSOCIATED INTERSTITIAL LUNG DISEASE

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Background: Interstitial lung disease (ILD) is a common extra-articular manifestation of rheumatoid arthritis (RA) and also the most common non-musculoskeletal manifestation of idiopathic inflammatory myopathies (IIM), including polymyositis, dermatomyositis and clinically amyopathic dermatomyositis.