Conclusions: Committed pro-inflammatory IL-17A-producing CCR6 +Th memory cells shift towards anti-inflammatory cells with functional regulatory capacities upon exposure to active vitamin D. This process can contribute to restoring the immunological balance and inhibiting synovial inflammation in RA.

Disclosure of Interest: None declared

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THU0049

DEVELOPMENT OF TFH-TH1 LIKE CELLS THROUGH EPIGENETIC MODIFICATION BY STATS FAMILY FACTORS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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Background: Systemic lupus erythematosus (SLE) is a prototype of autoimmune disease characterised by chronic immune activation and multiple immunologic phenotypes⁽¹⁾. Among several types of immune cells, T follicular helper (Tfh) cells serve important roles in the development and progression of SLE⁽²⁾.

Objectives: To assess the characteristics and mechanisms of differentiation of Tfh cells, we probed the phenotype of T helper cells in patients with SLE and underlying epigenetic modifications by cytokine-induced signal transducer and activators of transcription (STAT) family factors.

Methods: Naive CD4⁺ T cells and memory CD4⁺ T cells were isolated and stimulated by various cytokines and T cell receptor (TCR) *in vitro*. Expression of characteristic markers of Tfh-Th1-cells and phosphorylation of STATs were analysed by flow cytometry and qPCR. Histone modifications were evaluated by chromatin immunoprecipitation. Peripheral blood mononuclear cells from SLE patients and healthy controls were analysed by flow cytometry and productions of cytokines in serum were tested by cytometric bead array.

Results: Differentiation of CXCR5⁺CXCR3⁺Bcl-6⁺T-bet⁺IL-21⁺IFN- γ ⁺ Tfh-Th1-like cells was induced by IL-12. Among STAT family, STAT1 and STAT4 were phosphorylated simultaneously by IL-12 independent of IFN- γ and directly bound on Bcl-6 and T-bet gene loci accompanied by suppression of trimethylated histone 3 lysine 27. Compared with healthy controls, responsiveness of activation of STAT1 and STAT4 by IL-12 and proportion of activated Tfh-Th1-like cells were increased in patients with SLE.

Conclusions: Our findings suggest that IL-12-mediated co-activation of STAT1 and STAT4 alter histone modification, resulting in development of Tfh-Th1-like cells that are characteristically expanded in patients with SLE. These findings could be one of underlying pathogenesis of SLE and potentially helpful towards development of cell-specific treatment.

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Rheumatoid arthritis – etiology, pathogenesis and animal models

THU0050

HYPOXIA INDUCES PRODUCTION OF CITRULLINATED PROTEINS IN HUMAN FIBROBLAST-LIKE SYNOVIOCYTES THROUGH REGULATING HIF1A

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Background: Hypoxia is a prominent microenvironment feature in a range of disorders including cancer, rheumatoid arthritis (RA), atherosclerosis, inflammatory functions of bowel disease (IBD), infection and obesity. Hypoxia promotes biological functions of fibroblast-like synoviocytes via regulating hypoxia-inducible factor 1 α (HIF1 α). Dysregulated protein citrullination in RA drives the production of antibodies to citrullinated proteins, a highly specific biomarker of RA. However, the mechanisms promoting citrullination in RA are not yet fully elucidated.

Objectives: In the present study, we investigated whether pathophysiological hypoxia as found in the rheumatoid synovium modulates the citrullination in human fibroblast-like synoviocytes (HFLS).

Methods: HFLS were incubated in a hypoxic chamber (5% CO₂ and 1% O₂, balanced with N₂ as indicated) or in a normal incubator containing 5% CO₂ and approximately 20% O₂. The realtime quantitative PCR and western blotting were used to detect the expression of peptidylarginine deiminase 2, peptidylarginine deiminase 4 and citrullinated proteins. HFLS were transfected with HIF1 α siRNA to block the HIF1 α pathway.

Results: In the present study, we found that peptidylarginine deiminase 2 (PAD2) and citrullinated proteins were increased in HFLS after exposed to hypoxia. Moreover, knocking down HIF1 α by hif1 α siRNA ameliorated the expression of PAD2 and citrullinated proteins.

Conclusions: Collectively, the present study provide a new mechanism involved in generating citrullinated proteins: hypoxia promote citrullination and PAD production in HFLS. Concurrently, we also proposed a novel hypoxia involved mechanism in RA pathogenesis. The present study deepens our understanding of the role of hypoxia in the pathogenesis of RA and provides potential therapeutic strategy for RA.

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THU0051

TNF RECEPTOR 2 PLAYS AN IMMUNOREGULATORY AND ANTI-INFLAMMATORY ROLE IN ARTHRITIS

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Background: Despite the overall success of TNF α inhibitors in rheumatoid arthritis (RA), up to half of patients are classified as either primary or secondary non-responders¹. One hypothesis put forward to explain resistance to anti-TNF α therapy is an ascendant effect of dysregulated regulatory T cells and increased Th17 responses following TNF α blockade. Previous studies have demonstrated that TNFR2 is critical for stabilisation and suppressive function of regulatory T cells^{2,3}. However, TNFR2 also activates pro-inflammatory signalling cascades and, to date, the net effect of TNFR2 on the pathogenesis of RA remains unclear.

Objectives: In this study we address this question by assessing the progression of collagen-induced arthritis (CIA) in mice deficient for TNFR1 or TNFR2.

Methods: C57BL/6N.Q (H-2^d) mice were immunised with bovine type II collagen emulsified in complete Freund's adjuvant. The mice were monitored daily for arthritis and scored clinically from the day of onset of disease. Mice were culled on day 10 after arthritis onset and spleens, lymph nodes, serum and paws were collected for further analysis.

Results: As expected, TNFR1^{-/-} mice were found to be largely resistant to arthritis both clinically and histologically (figure 1). In contrast, there was significantly enhanced disease activity at the clinical and histological levels in TNFR2^{-/-} mice (figure 1) and this was accompanied by increased expression of the pro-inflammatory cytokines, TNF α and IL-6, reduced numbers of regulatory T cells, reduced FoxP3 expression and reduced expression of the immune inhibitory molecules, PD-1 and LAG3, in TNFR2^{-/-} mice compared to WT mice.

Abstract THU0051 – Figure 1. Progression of CIA in mice deficient for TNFR1 or TNFR2

Conclusions: This study has shown that TNFR2 signalling plays immunoregulatory and anti-inflammatory roles in CIA. First, it contributes to promotion of regulatory T cell generation and FoxP3 expression, and second, it limits the expression of pro-inflammatory cytokines. TNFR2 also regulates the expression immune inhibitory

molecules during inflammation. The results support the rationale to for development of TNFR1 specific antagonists or TNFR2 agonists for the treatment of RA.

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THU0052 A CIRCULATING PROTEIN SIGNATURE CORRELATES WITH SYNOVIAL PATHOTYPES IN RHEUMATOID ARTHRITIS PATIENTS

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Background: Rheumatoid arthritis (RA) is a disease characterised by high clinical variability and an underlying cellular and molecular heterogeneity. Efforts to find tools for the classification of the different disease phenotypes and patient stratification are essential to develop tailored therapies and improve its management. According to this, specific pathological phenotypes of synovial tissue (pathotypes) have emerged as associated with diverse clinical evolution and response to therapy.¹

Objectives: To identify signatures of circulating proteins associated with specific synovial pathotypes in RA patients.

Methods: A proteomic analysis was carried out on samples from patients enrolled in the Pathobiology of Early Arthritis Cohort. Ultrasound-guided synovial biopsies from these patients allowed their classification into three groups: lymphoid (L), myeloid (M) or fibroid (F), according to the pathotype. The study was performed using 54 serum samples at baseline. Sera were analysed by nanoliquid chromatography coupled to mass spectrometry using a SWATH strategy on a triple TOF (Sciex). The proteomic data were processed using ProteinPilot and PeakView. A two-stage support vector machine (TSSVM) with RBF kernel and 10 cross-fold validation for each meta-model was applied using the Classifyfire, e1071 and caret R packages.

Results: The proteomic analysis led to the identification and quantification of 229 proteins in all samples. A screening was performed on a group of 30 samples (Train set: 10 L, 10 M and 10 F). Data were pre-processed by PCA for dimension reduction. Then, application of machine learning tools led to the identification of a panel of 11 proteins whose different abundance is associated with a specific synovial phenotype (either L, M or F) in RA patients. As shown in the table 1, a very high accuracy and Kappa coefficient were achieved with this classification tool. The results were confirmed on an independent validation set of 24 samples (12 L, 8 M and 4 F) with also good performance. This protein signature allowed the correct classification of the samples into the three pathotypes with very high sensitivity and specificity (see table 1).

Abstract THU0052 – Table 1. Metrics of the classification performance of the 11-protein panel identified in this work as associated with the synovial pathotype of the patient. Cut-off for significance was p-value<0.05.

Train set				Classes in the train set			
				Fibroid		Lymphoid	
Accuracy	0.9667	95% CI	(0.8278–0.9992)	Sensitivity/	1.000/	1.000/	0.9091/
				Specificity	1.000	0.9524	1.000
				Pos/Neg	1.000/	0.9000/	1.000/
Kappa	0.95	P-Value	4.48E-09	Pred Value	1.000	1.000	0.9500
				Classes in the validation set			
Validation set				Fibroid		Lymphoid	Myeloid
Accuracy	0.875	95% CI	(0.6764–0.9734)	Sensitivity/	1.000/	0.8462/	0.8750/
				Specificity	0.9524	0.9091	0.9375
				Pos/Neg	0.7500/	0.9167/	0.8758/
Kappa	0.7907	P-Value	0.0005881	Pred Value	1.000	0.8333	0.9375

Conclusions: A signature of 11 circulating proteins has been identified as associated with synovial pathotypes in RA patients. The putative correlation of this signature with the clinical evolution and/or response to therapy of the patients remains to be elucidated.

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THU0053 ANTI-FRACTALKINE MONOCLONAL ANTIBODY AMELIORATES JOINT DESTRUCTION IN COLLAGEN-INDUCED ARTHRITIS MODEL BY INHIBITION OF OSTEOCLAST PRECURSOR CELL SURVIVAL AND MIGRATION

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Background: In the Phase 1/2 clinical study, E6011, a novel humanised anti-fractalkine (FKN) mAb demonstrated a promising efficacy in active RA patients who were inadequately controlled by MTX and/or TNF- α inhibitors (NCT 02196558). FKN is expressed on endothelial cells and fibroblast-like synoviocytes in synovium and also expressed on osteoblasts. CX3CR1 is expressed on monocytes/macrophages and osteoclast precursor cells (OPCs). Therefore, FKN-CX3CR1 interaction could play pivotal roles in migration, differentiation and activation of those cells. However, the precise mechanism(s) of FKN-CX3CR1 axis in RA, especially on joint destruction remains to be elucidated.

Objectives: We examined the roles of FKN-CX3CR1 axis in joint destruction, particularly focused on osteoclast precursor cells in *in vitro* and *in vivo* by using anti-mouse FKN mAb (anti-mFKN mAb).

Methods: DBA/1J mice were immunised with intradermal injections of bovine type II collagen to induce arthritis (CIA). Anti-mFKN mAb or control IgG were intraperitoneally injected twice a week. The clinical arthritis score was monitored, and joint destruction was evaluated by soft X-ray and histopathology. Plasma levels of joint destruction markers were assessed by ELISA. FKN expression in joint tissues were assessed by immunohistochemistry. Cell survival of bone marrow-derived OPCs without or with immobilised FKN was also assessed by FACS. In *in vivo*, OPCs were labelled by fluorescein and transferred to CIA mice to evaluate migration of OPCs into inflamed synovium. Anti-mFKN mAb or control IgG were injected before the cell transfer. The number of fluorescein-labelled OPCs that migrated into the CIA joint tissue were analysed.

Results: In both prophylactic and therapeutic treatments, anti-mFKN mAb clearly reduced the clinical arthritis score, soft X-ray score. Plasma levels of cartilage oligomeric matrix protein (COMP) and matrix metalloproteinase-3 (MMP-3) were also decreased after treatment with anti-mFKN mAb. Histological analysis revealed that anti-mFKN mAb inhibited synovitis, pannus formation, cartilage destruction, and that it suppressed bone damage with marked reductions in the number of tartrate-resistant acid phosphatase (TRAP)-positive cells. In *in vitro*, RANKL-induced osteoclast differentiation was enhanced by immobilised FKN, and anti-mFKN mAb suppressed FKN-dependent enhancement of osteoclast formation. FKN also enhanced cell survival of OPCs and eventually increased the number of OPCs. In *in vivo*, increasing expression of FKN was detected on endothelium in synovial tissue of CIA mice, and fluorescein-labelled OPCs migrated into the affected synovium. Anti-mFKN mAb clearly abrogated their migration into synovium.

Conclusions: Anti-mFKN mAb remarkably ameliorated the joint destruction with the marked reduction of osteoclasts by the inhibition of both OPC survival and OPC migration in inflamed joint tissues. These results strongly indicate that inhibition of FKN-CX3CR1 axis by a humanised anti-FKN mAb, E6011, is an attractive and affected joints-selective therapeutic strategy for the treatment of both inflammatory synovitis and joint destruction in RA patients.

Disclosure of Interest: None declared

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THU0054 LONG NON-CODING RNA GAPLINC PROMOTES PROLIFERATION AND INVASION OF FIBROBLAST-LIKE SYNOVIOCYTES AS MICRORNA SPONGING IN RA PATIENTS

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Background: Accumulating evidence suggested that long non-coding RNAs (lncRNAs) play diverse functional roles in many autoimmune diseases including