annexin V and propidium iodide apoptosis detection kit; autophagy was analyzed by western blot for the expression level of the autophagic marker LC3-II.
Results: Our results showed that MPs purified from RA patients at T0 expressed TNF on their surface and this expression decreased after three months of treatment with Etanercept (p=0.04). Moreover, serum RA-MPs at T0 significantly increased, in a dose-dependent manner, both apoptosis and autophagy levels in the human umbilical vein cell line *EA. hy926* (p=0.005 and p=0.02, respectively *versus* untreated cells). After three months of treatment with Etanercept, RA-MPs were not able to significantly change these parameters.Finally, in *vitro* studies showed that RA-MPs treated with Etanercept significantly decreased surface expression of TNF and were no longer able to modulate apoptosis and autophagy in *EA.hy926* cells.
Conclusions: Our data demonstrate that serum RA-MPs express TNF on their surface. Moreover, both in *vivo* and in *vitro* treatment with Etanercept interfere with the ability of MPs to significantly modulate apoptosis and autophagy of endothelial cells by decreasing surface expression of TNF.
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FRI0028 IN VITRO INHIBITORY EFFECT OF ETANERCEPT ON AUTOPHAGY: A NEW MECHANISM OF ACTION OF TNF INHIBITORS IN RHEUMATOID ARTHRITIS

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Background: Autophagy has emerged as a key mechanism in the development, survival and function of immune cells and dysregulation of autophagic pathway has been implicated in the pathogenesis of several autoimmune diseases including Rheumatoid Arthritis (RA) (1). In fact, autophagy seems to be involved in the generation of citrullinated peptides, with consequent breakage of tolerance in RA (2). Moreover, increased autophagy levels and a reduction of apoptosis-related molecules have been found in RA synovial tissues and a role of TNF-induced autophagy in RA development has been proposed (3).
Objectives: The aim of the study was to analysed the effect of TNF and anti-TNF inhibitor etanercept on autophagy and apoptosis in cells involved in RA pathogenesis.
Methods: Peripheral blood mononuclear cells (PBMCs) and fibroblast-like synoviocytes (FLS) isolated from RA patients were cultured in presence of TNF and in serum deprivation state (starvation) for 4 hours and then etanercept, at concentration of 15 ug/mL, were added to the culture. After 24h cells were analyzed for levels of autophagy marker LC3-II by western blot and for percentage of annexin V-positive apoptotic cells by flow cytometry.
Results: As expected, TNF and starvation induced autophagy on RA PBMC and FLS in dose-dependent manner after 24h of culture ($p<0.05$ in all experimental conditions). Moreover, the adding of etanercept caused a significant reduction of LC3-II levels (p=0.004) and an increase of apoptosis rate (p=0.002) after both pro-autophagic stimuli ($p<0.05$).
Conclusions: We demonstrated for the first time an inhibitory effect of etanercept on autophagy activation of cells involved in RA pathogenesis. In addition, our findings suggest a crucial role of autophagy in RA cells survival.
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FRI0029 THE OXYGEN SENSOR PHD1 IS AN INDISPENSABLE REGULATOR OF ARTHRITIS DEVELOPMENT

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Background: Oxygen supply is a fundamental requirement for all living tissues. Some tissues such as articular joints are characterized by a physiological state of hypoxia. Interestingly, under conditions of inflammation such as in arthritic disease, this level of hypoxia is even further enhanced. However, the functional significance of these observations and the molecular mechanisms involved remain poorly characterized to date. Our goal was therefore to examine the role of 3 known oxygen sensors, prolyl hydroxylase domain (PHD) proteins: PHD1, PHD2 and PHD3. They are enzymes whose function is essentially controlled by oxygen. Their expression pattern varies between either of them and all of them have been ascribed specific roles in a myriad of biological processes. [1]
Objectives: Our goal was to examine the role of oxygen sensors PHD1, PHD2 and PHD3 in preclinical models of rheumatoid arthritis, and to delineate the cellular source involved.
Methods: We subjected the collagen antibody induced arthritis (CAIA) model (resembling rheumatoid arthritis) to hypoxic (10% O_2) and normoxic conditions (21% O_2), respectively. Furthermore, the CAIA-model was induced in mice with germline deficiency of the specific PHD's and in mice with a myeloid cell-specific PHD1 deficiency versus controls. Arthritis development was assessed by clinical scoring of paw swelling, histopathology of knee joints and μCT.
Results: Mice kept in hypoxic conditions during CAIA experiments showed markedly less arthritis (both by clinical and histopathological assessment) compared to mice in normoxic conditions. Furthermore, we demonstrated that PHD1 knock-out (KO) mice had significantly less joint inflammation compared to wildtype mice. PHD1 KO mice were also protected against inflammation induced bone loss as evidenced by μCT. By contrast, no differences were found between PHD2 heterozygous (PHD2 KO mice are not viable) or PHD3 KO mice and littermate controls. Because myeloid cells are considered critical effector cells upon passive transfer of arthritogenic antibodies in the CAIA model we also generated myeloid cell specific ko mice ($PHD1^{myelKO}$). Of interest, $PHD1^{myelKO}$ mice developed less arthritis compared to wildtype mice and were protected against inflammation induced bone loss.
Conclusions: Our data are consistent with a new paradigm that the oxygen sensor PHD1 is a critical regulator of myeloid cell function in arthritic disease. Overall, the data suggest that PHD1 is a potential target in the treatment of arthritis.
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FRI0030 SYNOVIAL MAST CELLS IDENTIFY PATIENTS WITH A SEVERE PHENOTYPE IN A COHORT OF DMARD NAÏVE PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

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Background: Mast cells (MCs) are among the immune cells participating to the inflammatory response in Rheumatoid Arthritis (RA), but their exact contribution to disease development and progression is unclear.
Objectives: To evaluate the presence of MCs in the synovia of patients with early RA naïve to treatment and their correlation with baseline clinical phenotype, response to DMARDs and disease progression.
Methods: DMARD-naïve patients with early (<12 months) RA (n=99) fulfilling the 2010 ACR/EULAR criteria were recruited as part of the Pathobiology of Early Arthritis Cohort at Barts Health NHS Trust. Sections of paraffin embedded synovial tissue obtained by ultrasound-guided synovial biopsy were stained by immunohistochemistry for CD117 (c-kit) and patients were classified into MC+ and MC- groups. Differences in clinical parameters at baseline and 6 months and progression in radiographic damage at 12 months were evaluated. The expression of Receptor Activator of Nuclear factor Kappa-B Ligand (RANKL) by human primary MCs was assessed by western blot and immunofluorescence.
Results: The presence of synovial CD117+ MCs was significantly associated with highly active disease (DAS28, ESR, CRP, tender and swollen joint counts, $p<0.05$). At the 6 months follow-up, there were no differences in terms of response to treatment with synthetic DMARDs (e.g. DAS28 remission 38.8% in MC+ vs 42.9% in MC-, p=0.725). Nonetheless, MC+ patients showed a significantly higher prevalence of radiographic progression at 12 months (progressors/non progressors 13/38 in MC+ vs 0/28 in MC-, p=0.003, n=79). When progressors were compared with non progressors, there were no differences in clinical parameters at baseline (inflammatory markers, DAS28, ACPA or RF positivity). Because of the observed association of MCs with radiographic progression, we looked for mechanisms that could link MCs to bone erosions, and we found that human primary MCs express the osteoclast activator RANKL.
Conclusions: We show that synovial MCs identify patients with a severe clinical phenotype in a DMARD-naïve early RA cohort. In particular, despite a similar rate of response to DMARDs at 6 months, the presence of synovial MCs at baseline was significantly associated with radiographic progression at 12 months. As clinical parameters at baseline showed no association with radiographic

progression, our work suggests that the analysis of synovial MCs could help to identify patients at high risk of progression in radiographic damage, warranting further investigations to confirm the association of MCs with the development of joint damage and their direct contribution to bone erosion, possibly via a RANKL-mediated activation of osteoclasts.

Disclosure of Interest: None declared

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FRI0031 DNA OXIDASE ENZYME TET3 EXACERBATES SYNOVIAL INFLAMMATION AND BONE DESTRUCTION

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Background: In rheumatoid arthritis (RA), fibroblast-like synoviocytes (FLSs) play an important role in joint destruction. We have shown a disease-specific DNA methylation pattern in RA patient-derived FLSs (RA FLSs). In 2009, active demethylation enzymes, Ten-Eleven translocation (TET) 1/2/3, were demonstrated. However, little is known about the role of the TET protein family in RA FLSs

Objectives: The aim of this study was to examine the role of the TET protein family in the pathological process of RA from an *in vivo* perspective with a mouse model, and from an *in vitro* perspective using human synovial tissue.

Methods: K/BxN serum-induced arthritis was induced in *Wild-type* (*WT*) and TET3 heterozygous-deficient (*TET3*$^{+/-}$) C57BL/6 mice. Synovial tissues were obtained from patients with RA and Osteoarthritis (OA) who had received joint replacement surgery. FLSs were transfected with siRNA and knocked down (KD). Gene expression was determined by qPCR and protein expression by western blot and immunostaining. 5-hydroxymethylcytosine (5hmC) was determined by dot blot and hMeDIP assay. Cell migration were assessed using a scratch assay.

Results: TET3 was expressed in particularly infiltrative synovial areas in K/BxN-*WT* mice. The arthritis score of K/BxN-*TET3*$^{+/-}$ was not increased after day 8. Histologically, synovial inflammation and proliferation, and bone destruction were significantly suppressed in the K/BxN-*TET3*$^{+/-}$ mice. In the RA synovial membrane, TET3 was more highly expressed in RA than in OA. In cultured FLSs, the expression of *TET3* mRNA tended to be slightly higher in RA than in OA. After 2 h of stimulation with various cytokines, the expression level of *TET3* mRNA was increased by stimulation with TNFα, interleukin (IL)-1, or IL-17. Assessment of the protein expression of TET3 after stimulation by TNFα using extracted intranuclear proteins of FLSs showed an increase in the expression level of TET3 protein over time. Similarly, the expression level of 5hmC was increased after stimulation by TNFα. Next, the function of TET3 in the FLSs was examined using TNF stimulation follwoing TET3-KD. A scratch assay of cell migration and invasion demonstrated that the TNFα-dependent migration and invasion of FLSs was completely inhibited by TET3-KD. The protein levels of CCL2 were strongly induced by TNFα stimulation, and inhibited by TET3-KD. In addition, flow cytometry of the expression of intercellular adhesion molecule-1 (ICAM-1) revealed that the expression of ICAM-1 was TNFα-dependent and was inhibited by TET3-KD. Assessment of gene expression changes showed that the induction of TNFα-dependent *CCL2* and *ICAM-1* mRNA expression was significantly inhibited by TET3-KD. Subsequently, although 5hmC level on the *ICAM-1* promoter was increased by TNF stimulation, it was strongly inhibited by TET3-KD.

Conclusions: *In vivo*, the arthritis and bone erosion were significantly decreased in the *TET3*$^{+/-}$ mice. *In vitro*, TET3 was induced by inflammatory cytokine stimulation, and TET3 knockdown inhibited the cytokine-induced expression of CCL2 and ICAM-1 in RA FLSs. In addition, hydroxymethylation of the ICAM-1 promoter region was dependent on TET3. These results suggest that continuous exposure to inflammatory cytokines results in leaving an inflammatory memory in FLS in a TET3-dependent manner, thereby promoting pannus formation and increasing the probability of joint destruction.

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FRI0032 CURATIVE EFFECT OF CAMELLIA SINENSIS (CS) AGAINST OPPORTUNISTIC INFECTION IN VULNERABLE ANIMAL MODEL OF RHEUMATOID ARTHRITIS

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Background: Rheumatoid arthritis (RA) is an autoimmune disease characterised by chronic inflammation of pro-inflammatory cytokines. Opportunistic infection plays a significant role in loss of tolerance to citrullinated proteins along with inflammatory progression of RA. Due to the immunosuppressive property of anti-rheumatic drugs, the patients of RA become highly vulnerable to microbial infections [1]. Thus, the present study employed an *in vivo* animal model to explore the holistic remedies for the effective treatment of RA.

Objectives: To study the immunomodulatory effect of *Camellia sinensis (Cs)* against inflammatory disorder

Methods: Study utilized collagen induced arthritis (CIA) rat model with *Salmonella typhimurium* (10^8 CFU/ml, p.o) as an opportunistic infectious agent which was introduced to enhance disease severity (on 21st day)[2]. Treatment with Cs at oral dose 400 mg/kg/body wt. (p.o) was started from 21st day for 14 days to explore its curative, anti-edematogenic effect and quantitation of oxidative stress markers. To validate biochemical changes, the histopathologiy and level of cytokines were also studied in joint tissue followed by 7 Tesla Magnetic Resonance Imaging (7T MRI).

Results: Treatment groups significantly restored the level of oxidative stress markers (Table-1). Furthermore, there was significant reduction in the number of bacterial colonies in blood and fecal matter in the treatment group as compared to infected group, while pro-inflammatory cytokine level of TNF-α, IL-1, IL-6 was significantly lower in joint tissue. Histological & 7T-MRI changes in the treatment group included significant reduction of cartilage erosion & pannus formation and there were no signs of inflammation in the small intestine as compared to arthritic and infected group (Figure 1).

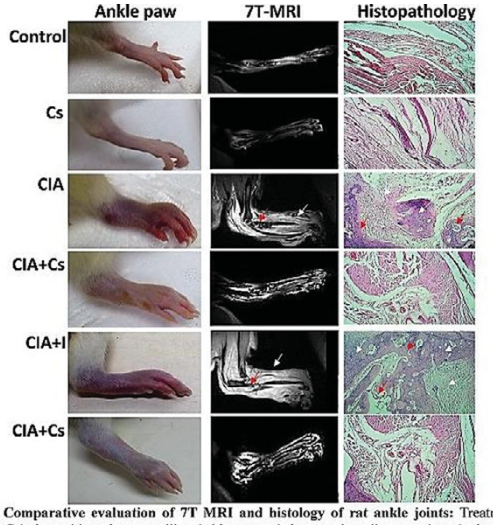

Fig 1: Comparative evaluation of 7T MRI and histology of rat ankle joints: Treatment group (CIA+Cs, CIA+I+Cs) showed less tissue swelling (white arrows), bone and cartilage erosions (red arrows) as compared to infected groups (CIA , CIA+I) and there was no significant change in between control and Cs only groups.

Conclusions: The present study demonstrated that Cs has anti-inflammatory effect and could also be used as potent immunomodulator to manage RA.

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Abstract FRI0032 – Table 1. Effect of Cs on oxidative stress markers

S. No	Control	Cs	CIA (Arthritic)	CIA+Cs (Treatment)	CIA+I (Infected)	CIA+I+Cs (Treatment)
Glutathione (μmoles of GSH/g tissue)	1.29±0.042	1.69±0.02	0.51±0.26*	1.40±0.06##	0.56±0.032*	1.45±0.034#
Lipid peroxidation (μmoles of TBARS formed/hr/g tissue)	1.40±0.0095	1.33±0.023	2.09±0.073*	1.80±0.055##	2.32±0.043*	1.73±0.073##
Articular elastase (ng/g protein)	135±5.00	136±4.50	250±0.13*	182±1.00##	242±0.3*	193±2.5##
Superoxide dismutase (nmoles of epinephrine protected from oxidation /min/mg protein)	2.20±0.085	2.21±0.082	1.84±0.135*	2.35±0.80#	1.69±0.05*	2.63±0.1##
Nitric oxide (μmoles nitrite/mg wet tissue)	0.44±0.03	0.39±0.065	0.97±0.1*	0.66±0.2#	0.95±0.4*	0.73±0.03#
Catalase (μmoles of H2O2 consumed/min/mg protein)	161±2.94	144±3.10	39±0.005*	106±1.00##	37±2.00*	82±1.50##

All the values expressed in Mean ± SD (n=6); Significant differences indicated by #p<0.05 and ##p<0.01 as compared to CIA and CIA+I group and *p<0.001 as compared to control group.