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.
References:
[1] Beyer C and Pisetsky DS.The role of microparticles in the pathogenesis of rheumatic diseases. Nat. Rev. Rheumatol. 2010;6:21–9.
[2] Feldmann M, Brennan FM, Foxwell BM and Maini RN.The role of TNF alpha and IL-1 in rheumatoid arthritis Curr. Dir. Autoimmun. 2001;3:188–99.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.3434

FRI0028 IN VITRO INHIBITORY EFFECT OF ETANERCEPT ON AUTOPHAGY: A NEW MECHANISM OF ACTION OF TNF INHIBITORS IN RHEUMATOID ARTHRITIS

M. Vomero[1], A. Capozzi[2], C. Barbati[1], T. Colasanti[1], V. Manganelli[2], F. Ceccarelli[1], F.R. Spinelli[1], F. Conti[1], C. Perricone[1], A. Finucci[1], M. Pendolino[1], R. Scrivo[1], R. Misasi[2], M. Sorice[2], G. Valesini[1], C. Alessandri[1]. *[1]Department of Internal Medicine and Medical Specialties, Arthritis Center, Sapienza University of Rome; [2]Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy*

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.
References:
[1] Pierdominici M, Vomero M, Barbati C et al. Role of autophagy in immunity and autoimmunity, with a special focus on systemic lupus erythematosus. FASEB J. 2012; 26:1400–12.
[2] Sorice M, Iannuccelli C, Manganelli V et al. Autophagy generates citrullinated peptides in human synoviocytes: a possible trigger for anti-citrullinated peptide antibodies. Rheumatology. 2016;55:1374–85.
[3] Rockel JS, Kapoor M. Autophagy: controlling cell fate in rheumatic diseases. Nat Rev Rheumatol. 2016;12:517–31.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.5061

FRI0029 THE OXYGEN SENSOR PHD1 IS AN INDISPENSABLE REGULATOR OF ARTHRITIS DEVELOPMENT

K. De Wilde[1,2], D. Gaublomme[1,2], J. Coudenys[1,2], F. Windels[1,2], S. Van Welden[3], A. De Muynck[4], D. Elewaut[1,2]. *[1]Department of Rheumatology, Department of Internal Medicine, Ghent University; [2]Unit for Molecular Immunology and Inflammation, VIB Inflammation Research Center; [3]Department of Gastroenterology; [4]Department of Physics and Astronomy, Ghent University, Ghent, Belgium*

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.
References:
[1] Fong G-H, Takeda K. Role and regulation of prolyl hydroxylase domain proteins. Cell Death Differ 2008;15:635–41. doi:10.1038/cdd.2008.10.
Disclosure of Interest: None declared
DOI: 10.1136/annrheumdis-2017-eular.5194

FRI0030 SYNOVIAL MAST CELLS IDENTIFY PATIENTS WITH A SEVERE PHENOTYPE IN A COHORT OF DMARD NAÏVE PATIENTS WITH EARLY RHEUMATOID ARTHRITIS

F. Rivellese[1], F. Humby[1], A. Nerviani[1], S. Pagani[1], D. Mauro[1], A. de Paulis[2], G. Marone[2], C. Pitzalis[1]. *[1]Centre for Experimental Medicine & Rheumatology, William Harvey Research Institute, Barts and the London School of Medicine & Dentistry, London, United Kingdom; [2]Department of Translational Medical Sciences (DiSMeT) and Center for Basic and Clinical Immunology Research (CISI), University of Naples Federico II, Naples, Italy*

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