tis (OA) is driven by a low-grade inflammation in the synovium. Pro-inflammatory cytokines released locally during OA, such as S100A8/A9 which are expressed for prolonged periods when compared to IL-1β, IL-6, and TNF-α, may recruit monocytes from the bone marrow (BM) to the joint. In mice, two functionally distinct monocyte populations are described: (i) pro-inflammatory Ly6C$^{high}$ monocytes; and (ii) patrolling Ly6C$^{low}$ monocytes.
**Objectives:** The objective of our study is to investigate the role of S100A8/A9 in the recruitment of the different monocyte populations during early collagenase-induced OA (CiOA).
**Methods:** S100A8 was intra-articularly injected into the knee joint of naïve wild type C57BL/6 (WT) mice to investigate their direct role in recruitment of monocytes. CiOA was induced by unilateral intra-articular collagenase injection in WT, and S100A9KO mice to investigate the role of S100A8/A9 in more detail. At CiOA day 7, WT and S100A9KO mice were sacrificed together with age-matched saline-injected control mice (n=6/group), and expression of several monocyte cell markers, chemokines, and adhesion molecules were measured in the synovium and BM, in which the cells were also analyzed by FACS. Monocytes were identified as (B220/CD90/CD49b/NK1.1/Ly6G)$^{low}$CD11b$^{high}$(F4/80/MHCII/CD11c)$^{low}$, which were further divided into subsets based on their Ly6C expression.
**Results:** Injection of S100A8 into the knee joints of naïve mice led to a significantly elevated expression of monocyte-related markers (Ly6C, CCR2, and CX3CR1) and monocyte attracting chemokines (MCP-1, CX3CL1, MIP1α, and KC) within the synovium after 1 and 3 days, suggesting that S100A8/A9 is directly involved in the attraction of monocytes. At CiOA day 7 in WT mice, numbers of Ly6C$^{high}$, but not Ly6C$^{low}$ monocytes, were strongly increased (7.6-fold) in the synovium as compared to saline-injected control joints. In contrast, S100A9KO mice showed a significant increase in Ly6C$^{low}$ monocytes (2-fold), whereas the number of Ly6C$^{high}$ monocytes remained unaffected. Concurrently, a strong upregulation of several chemokines (MCP1, CX3CL1, KC, and MIP1α) was observed locally in the synovium, of which only the Ly6C$^{low}$ mobilization marker CX3CL1 was significantly higher in S100A9KO mice, corresponding with the increased Ly6C$^{low}$ monocytes in the synovium of S100A9KO mice. This could however not explain the local increased number of Ly6C$^{high}$ monocytes at CiOA day 7 in WT mice, and therefore we next investigated the main source of the monocytes, which is the BM. We observed a decrease of 14% of myeloid cells (consisting partly of Ly6C$^{high}$ monocytes) in the BM of WT mice at CiOA day 7, whereas there were no changes in the BM of S100A9KO mice, suggesting that S100A8/A9 affects the release of myeloid populations from the BM. In line with that, expression of adhesion molecules (LFA-1, VCAM, VE-cadherin, PECAM1, and L-selectin) was lower at CiOA day 7 in the BM of S100A9KO mice when compared to WT mice.
**Conclusions:** Local induction of OA leads to S100A8/A9 production, and subsequently to the mobilization of Ly6C$^{high}$ monocytes into the joint, driving OA pathology.
**Disclosure of Interest:** None declared
**DOI:** 10.1136/annrheumdis-2017-eular.6181

## SAT0013 THE PATHOGENIC ROLE OF MYELOID CD141+ DENDRITIC CELLS IN INFLAMMATORY ARTHRITIS

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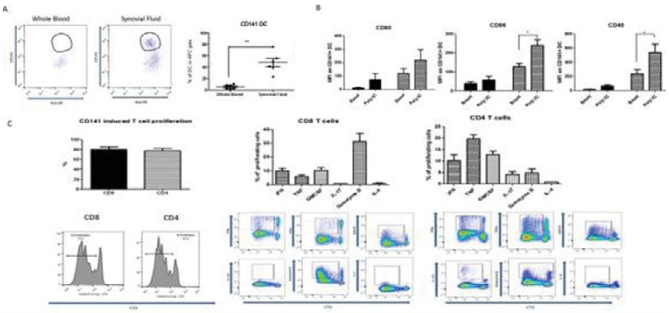
**Background:** Dendritic cell (DC) are a heterogeneous group of antigen presenting cells that can be subdivided into CD1c$^+$ & CD141$^+$ DC. CD141$^+$ DC are a rare population of DC that were first discovered in 2010 in human peripheral blood. Due to their rarity very little is known about the function of these cells in other tissue in or indeed disease. These newly described DC subset have thus never been described in Inflammatory Arthritis (IA) or any of the rheumatic diseases.
**Objectives:** To identify CD141 DC in IA synovium and functionally assess if these cells play a pathogenic role in IA.
**Methods:** CD141$^+$DC were magnetically purified from synovial fluid mononuclear cells (SFMC) and peripheral blood mononuclear cells (PBMC) stimulated and stained with a panel of fluorochrome conjugated antibodies for multicolour flow cytometry. CD141$^+$ DC isolated and purified from IA synovial fluid were subsequently cocultured with allogenic CD3$^+$ T cells for 6d after which intracellular cytokine production was assessed by flow cytometry. Supernatants from this DC-T cell cocultures were used to treat synovial fibroblasts & the expression of adhesion molecules, cytokines & MMPs was measured. Finally using sorted populations of CD141$^+$ DC from SFMC and PBMC, RNA sequencing was performed and differentially expressed genes and interaction network analysis were identified using the DeSeq2 R package, Ingenuity® Pathway Analysis (IPA) and InnateDB and Cytoscape.
**Results:** Within IA synovial fluid (SF), CD141$^+$ DC are significantly enriched compared to WB & express higher levels of the costimulatory activation markers CD80 CD86 and CD40. Following coculture of these SF CD141$^+$ DC with CD3$^+$ T cells, CD141$^+$ DC induce both CD8$^+$ & CD4$^+$ T cell proliferation. SF CD141$^+$ DC induce Granzyme B production from CD8$^+$ T cells & TNFα, IFNγ & GMCSF from CD4$^+$ T cells. The IA synovium consists of a complex interplay of multiple cell types. Therefore next we examined the effect of this CD141$^+$ DC-T cell interaction on the key invasive cells in the synovium – synovial fibroblasts. Supernatants from CD141 activated T cells were cultured with fibroblasts & induced expression of ICAM-1, IL-6, IL-8, MMP1 & MMP3. SF CD141 expressed significantly higher levels of the the hypoxia marker TREM1, activation of which induces further expression of CD80, CD86 and CD40. Coculture of these TREM1 activated CD141 with CD3$^+$T cells increases IFNγ and IL-17a production. Finally RNASeq analysis revealed that there are 2089 differentially expressed genes between SF CD141$^+$DC & WB CD141$^+$DC. These genes are involved in a number of key pathways such as energy metabolism, chemokine & cytokine signalling. Principal Component Analysis (PCA) revealed that CD141$^+$ DC with the synovium are distinctly different from blood CD141$^+$ DC



A) CD141 DC are enriched in the IA synovium compared to peripheral blood. B) IA CD141 from synovial fluid (SF) express higher expression of activation markers and respond to TLR engagement. C) CD141 DC isolated from IA SF induce CD4+ and CD8+ T cell proliferation and cytokine secretion

**Conclusions:** CD141$^+$ DC are enriched in the IA joint in an active state. RNASeq analysis revealed they are distinct from blood CD141$^+$DC and our *in vitro* data would support the hypothesis that these CD141$^+$DC contribute to synovial inflammation and joint destruction.
**Disclosure of Interest:** None declared
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## SAT0014 THE MUTATED RNA SPLICING PROTEIN HNRNP-A3 IS A NOVEL AUTOANTIGEN IN SYSTEMIC RHEUMATIC DISEASES A LINK TO WARBURG EFFECT IN RA

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**Background:** The present study was undertaken to investigate novel anti-hnRNPs autoantibodies in rheumatic diseases.
**Objectives:** Novel mutated hnRNP A3 was cloned out of RA synovial tissue linking it directly to Warburg effect and lactate production in RA. Increased lactate production in RA synovia and tumors is klinked to alternative splicing process from PKM1 to PKM2 hnRNPs dependent.
**Methods:** After immunobloting and 2D-gel-eletrophoresis of a semipurified hnRNP fraction two protein spots were sequenced and identified to be highly similar to hnRNPA3. The hnRNP A3 variants were cloned from RA synovial tissue, which identified the isoforms found on protein level. 3700 RA sera were screened for the presence of mutated anti-hnRNP A3 autoantibodies using recombinant proteins and peptides thereof. Binding of RNA to hnRNP A3 (MA3) and mutated citrullinated A3 peptides (MCA3) the epitope recognition was investigated. Expression of hnRNP A3 in synovial tissue was analysed by
**Results:** Autoantibodies to MA3 protein were detected in 13% of RA (n=215) patients, in 9% SLE (n=154), in 27% of MCTD patients (n=44/10) and in less than 5% of 129 patients with other rheumatic disorders but not at all in healthy controls on immunoblot. When using renaturated MA3 on ELISA 22% of RA patients were detected and 87% of these patients had erosive arthritis. Same modification as in cancer cells were identified in synovial tissue and verified by MS and DNA sequencing. Using 2–3 citrullinated MCA3 peptides up to 81% of patients (n=150) with established and 67% (n=2926) of patients with an early RA with a specificity of 97% were detected. In early RA 27% and 25% in established RA of CCP2 negative and 93% of CCP2 positive patients were identified.
By combining with the already established CCP2 and the new MCA3, 72% of early patients are positive. MCA3 autoantibodies predominantly occur ($p<0.001$) in an erosive, severe course of disease and approximately 60% of these patients receive a TNF alpha blocker within a year. MRL Lpr/lpr sera were hnRNP-A3 reactive and the antibody generation is Toll 7 and 9 dependent. Anti-hnRNP-A3-antibodies are directed to conformational RNA binding epitopes. Expression of hnRNP-A3 revealed the antigen to be overexpressed in RA synovial tissue.
**Conclusions:** Mutated hnRNP-A3 is as a novel Toll7/9 dependent autoantigen in systemic rheumatic diseases. These mutated proteins are major components of RNA and DNA containing alternative splicing complexes leading to the Warburg effect and and autoantibodies predominantly occurre in an erosive, and severe courses of RA.

**Disclosure of Interest:** None declared
**DOI:** 10.1136/annrheumdis-2017-eular.3647

### SAT0015 ANCA-ASSOCIATED VASCULITIS- AND SYSTEMIC LUPUS ERYTHEMATOSUS-INDUCED NEUTROPHIL EXTRACELLULAR TRAPS HAVE INTRINSICALLY DIFFERENT FEATURES

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**Background:** Neutrophil extracellular traps (NETs) are immunogenic, extracellular DNA structures that harness important autoantigens to be recognized by the adaptive immune system. NETs are thought to play a pivotal role in the pathogenesis of many systemic autoimmune diseases including ANCA-associated vasculitis (AAV) and systemic lupus erytematosus (SLE). However it is still unclear how and if NETs can act as a common pathway in the pathophysiology of these clinically divergent autoimmune diseases.
**Objectives:** To investigate the characteristics of NETs induced by sera of AAV and SLE patients.
**Methods:** The present study involved 101 AAV patients, 59 SLE patients and 10 healthy controls. Healthy neutrophils were stimulated with 10% serum of these patients to induce NETs. Quantity of NET induction was measured by a novel, highly-sensitive NET quantification assay using 3D-confocal laser scanning microscopy[1]. Qualitative characteristics of NETs were investigated by immunofluorescence microscopy that detected co-localisation of several established autoantigens and NET-markers on AAV- and SLE-induced NETs, including citrullinated histon-3 (CitH3), neutrophil elastase (NE), high mobility group box-1 (HMGB1), myeloperoxidase (MPO) and proteinase-3 (PR3). Additionally, the morphology and kinetics of AAV- and SLE-induced NETosis were visualized by live imaging and electron microscopy.
**Results:** Quantifying ex vivo NET induction demonstrated that AAV sera induced significant more NETs (median [Q1 - Q3]: 20.74 [9.56 – 74.14]), compared to SLE sera (5.02 [1.88 – 14.33]). Also qualitatively, NETs induced by AAV or SLE sera were distinct. In both cases, NETs showed co-localisation of MPO and PR3 with extracellular DNA. However, AAV-induced NETs had significantly higher Cith3 expression than SLE-induced NETs. Interestingly, the opposite was observed for other markers as HMGB1 was exclusively expressed on SLE-induced NETs and NE was also higher expressed on SLE-induced NETs compared to AAV-induced NETs. Intriguingly, the distinction between AAV and SLE NETs was further corroborated by live imaging demonstrating differences in morphology and chronology of NET induction: in SLE NET-clusters were induced within the 1st hour while in AAV non-clustered NETs composed of long, thin DNA-fibres were induced in 2–4 hours through lytic expulsion.
**Conclusions:** The present study demonstrates that NET induction in AAV and SLE results in quantitative and qualitative distinct NETs indicating that NET formation in AAV and SLE is likely based on intrinsically different processes. These data increase our understanding of the pathophysiologic relevance of NETs and how they could be considered as a common pathway underpinning different autoimmune diseases.
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**Disclosure of Interest:** None declared
**DOI:** 10.1136/annrheumdis-2017-eular.3804

### SAT0016 A NEW SUBSET OF NK CELLS, WITH ENHANCED CYTOTOXIC FUNCTION, IS INCREASED IN SYSTEMIC LUPUS ERYTHEMATOSUS PATIENTS

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**Background:** Natural killer cells (NK cells) are granular lymphocytes that belong to innate immunity, its major function is the lysis of virus-infected or tumoral cells. These functions are regulated by activating (NKG2D, NKp46, NKP30, NKG2C, CD161) and inhibitory receptors like KIRs and NKG2A. The role of NK cells in autoimmunity is poorly understood; it is well known that in systemic lupus erythematosus (SLE) patients there are decreased levels of NK cells. NK cells can modulate the adaptive immunity through its interaction with dendritic cells (DCs); the activation or killing of DC by NK cells depends of the ratio NK:DC. However, the interactions NK:DC in SLE have not been well studied. A new subset of NK cells with DCs characteristics has been reported in a model of lupus mice; however, the possible increment of NK cells with DC like phenotype in SLE has not been reported.
**Objectives:** To analyze the phenotype of circulating NK cells as well as its function in SLE patients.
**Methods:** Sixty SLE patients and fifty-five controls were included in this study. Diagnosis was made according to ACR criteria. Activity of disease was measured by SLEDAI index. The expression of NKG2A, ILT2, NKG2D, NKG2C, NKp30, NKp46, CD161, CD134, CD80, CD86, HLA-DR, CD11c+ was evaluated in NK cells (CD3-CD56+) from peripheral mononuclear cells. NK cell function was assessed by the percentage of monocyte-derived DC lysis by NK cells.
**Results:** Diminished levels of circulating NK cells were found in SLE patients ($p=0.0439$) compared to healthy subjects. NK cells from SLE showed higher levels of the inhibitory receptor ILT2+($p=0.0024$), and the costimulatory molecules CD86+ ($p=0.0136$) and CD134+($p=0.0238$); in addition, SLE patients displayed a higher expression of MHC-class II molecule, HLA-DR+ ($p<0.0001$). Interestingly, higher levels of atypical NK cells CD11c+HLA-DR+ ($p=0.0075$) were found in SLE patients compared with healthy subjects. Furthermore, we found that SLE patients showed a significate increased level of monocyte derived-DC lysis by NK cells.
**Conclusions:** In this study, we show for the first time that NK cells in SLE have an altered phenotype, expressing receptors, which are characteristic of dendritic cells (CD134, CD86 and HLA-DR). The expression of these receptors may provide NK cells with the ability to activate T cells, which together with their higher capacity to lyse immature or tolerogenic DCs could contribute to SLE pathogenesis. It is known that NK cells could have a dual role in autoimmune diseases, here we propose that the lysis of DC mediated by NK cells could be important to modulate the disease activity in SLE patients. Even more, in this report we identify a new subset of NK cells, CD11c+ HLA-DR+, reported previously in a mouse lupus model. It is essential to highlight that these NK cells with DC-like phenotype could be crucial for the development of SLE.
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**Disclosure of Interest:** None declared
**DOI:** 10.1136/annrheumdis-2017-eular.3565

**SATURDAY, 17 JUNE 2017**

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

### SAT0017 INVESTIGATING NOVEL AUTOANTIBODIES IN PATIENTS WITH GRANULOMATOSIS WITH POLYANGIITIS

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**Background:** Granulomatosis with polyangiitis (GPA) is a disease characterized by inflammation in small blood vessels, leading to significant organ damage and ultimately great morbidity and mortality. The presence of anti-neutrophil cytoplasmic antibodies (ANCAs) is a hallmark of the disease, and thought to directly play a role in pathogenesis by activating primed neutrophils. The two major antigens that ANCAs are believed to recognize are myeloperoxidase (MPO) and proteinase 3 (PR3). Although anti-MPO antibodies are directly pathogenic when transferred or induced in animal models, the evidence is less clear for PR3. Clinically, it is debated whether levels of anti-PR3 correlate with disease activity. Therefore, we believe that anti-PR3 antibodies are not the primary drivers of disease in patients with PR3+ ANCA-vasculitis (i.e. GPA), and that novel autoantibodies may play a role in disease pathogenesis.
**Objectives:** The objective of this work is to identify the human autoantigen targets of novel autoantibodies identified in GPA, and to test their association with disease. The goal is to discover novel autoantigens that will ultimately lead to new diagnostics and therapeutics.
**Methods:** Cell-barcode-enabled antibody repertoire sequencing was performed on blood plasmablasts (antibody-producing cells formed during an immune response) from five PR3+ ANCA-vasculitis patients with GPA treated with rituximab in the RAVE trial. Phylogenetic trees were bioinformatically created in order to identify clonal families of plasmablasts. Antibodies representing clonal families of plasmablasts were recombinantly expressed and are being tested using human protein arrays and ELISAs to determine their antigen specificity.
**Results:** All five PR3+ ANCA-vasculitis patients sequenced from the RAVE trial achieved complete remission but subsequently flared. Plasmablasts were isolated at the baseline flare, at remission and at the post-rituximab flare. Phylograms of the antibody repertoires revealed clonal families of affinity-matured antibodies that share heavy and light chain VJ usage. A total of 24 representative antibodies were selected for recombinant expression, including representative antibodies derived from clonal families: (1) shared across patients at baseline flare and/or post-rituximab flare (n=5), (2) present at baseline flare and post-rituximab flare (n=7) [persistent clone or same clone came back], (3) present at baseline flare or post-rituximab flare (n=6), (4) present in remission and post-rituximab flare (n=5) [achieves remission despite clone presence], (5) present in remission (but not flare) (n=1) [patient in remission despite clone presence]. None of the 24 antibodies bind PR3 in an ELISA and thus these antibodies do not represent