components such as dsDNA or nucleosomes was shown to play an important role in breaking immune tolerance against nuclear antigens.¹ Although the function of HMGB1 is in-part determined by the complexes it forms with other molecules, structural modifications in the HMGB1 polypeptide that may regulate complex formation have not been described.

Objectives: In this study we investigated the presence of HMGB1 in large protein complexes (HMGB1c) in human plasma. The objectives of this study were to isolate and characterise HMGB1c as well as to determine the mechanism of its formation.

Methods: We examined the presence of HMGB1c in plasma from SLE patients and healthy controls using semi-denaturing detergent agarose gel electrophoresis (SDD-AGE) followed by Western blot analysis. Immunoblotting, coimmunoprecipitation, confocal microscopy, and mass spectrometry were used to detect and characterise novel high molecular weight HMGB1 variants *in vitro* as well as in cell lines and primary cells. Mechanisms of HMGB1c formation were delineated via mass spectrometry, RNA interference, and *in vitro* enzyme reactions.

Results: In this study we note the presence of high molecular weight, denaturing resistant HMGB1 protein complexes (HMGB1c) that were present in the plasma of SLE patients and to a much lesser extent, healthy subjects. Similarly, HMGB1c were induced when cells were incubated with endotoxin or alum. Here we report that HMGB1c formation is catalysed by the calcium-activated protein crosslinking enzyme transglutaminase-2 (TG2). HMGB1-TG2 interaction was demonstrated via coimmunoprecipitation as well as by confocal microscopy after co-transfection of cells with plasmids encoding fluorescent-tagged HMGB1 and TG2 constructs. Moreover, HMGB1c formation was suppressed in cells by TG2 siRNA. Crosslink site mapping and analysis by mass spectrometry revealed that HMGB1 can be crosslinked to TG2 as well as a number of additional proteins, including human autoantigens.

Conclusions: TG2 catalyses the formation of high molecular weight HMGB1 protein complexes. Given the immunoadjuvant properties of HMGB1 and the implication of TG2-mediated protein complex formation as a possible mechanism by which immune tolerance can be broken to self-molecules,² these findings have significant physiological implications for the role of HMGB1 in cellular stress responses and innate immunity in lupus.

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Cytokines and inflammatory mediators

SAT0016 IL-12 SUPPRESS TR1 CELLS IN THE SJÖGREN'S SYNDROME

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Background: Sjögren's syndrome (SS) is a systemic autoimmune disorder characterised by lymphocytic infiltration of salivary and lacrimal gland leading to dry mouth and eye dryness progressively. It is known that pro-inflammatory cytokines and dysfunction of immune cells play an important role in the pathogenesis of SS. The regulatory T cell type 1 (Tr1) cells have strong immunosuppressive properties and alleviate inflammatory diseases. However, the role of Tr1 cells in SS and the underlying mechanism is still not fully understood.

Objectives: This study aims to determine the association of Tr1 cells with the disease pathogenesis using a murine model of SS and patients with SS.

Methods: The frequencies of Tr1 cells in blood, cervical lymph nodes and their association with disease severity were determined in SS mice. Since proinflammatory interleukine 12 (IL-12) have the ability to regulate the generation of Tr1 cells, the serum IL-12 and its association with disease severity and Tr1 cells were also examined. The involvement of IL-12 and a Tr1 response was examined by recombinant IL-12 treatment or anti-IL-12 antibody neutralisation in SS mice and

monitored for SS development. The mouse naive CD4⁺ cells were treated with IL-12 under Tr1 polarising condition. The frequency of Tr1 cells and their association with IL-12 and disease activity were verified in SS patients.

Results: Tr1 cells significantly decreased in SS mice and patients with SS, which correlated negatively with disease activity and proinflammatory IL-12 (figure 1A, B). IL-12 suppressed Tr1 cell polarisation *ex vivo*. SS mice treated with the recombinant IL-12 displayed significantly lower saliva flow rates and pronounced inflammation and tissue damage in salivary glands, accompanying with reduction of Tr1 cells. However, anti-IL-12 antibody treatment profoundly increased the numbers of Tr1 cells and ameliorated the reduction in salivary secretion and inflammation and tissue damage in salivary glands in SS mice (figure 1C).

Conclusions: Our findings indicate that proinflammatory IL-12 with its capacity to inhibit Tr1 cell generation may be a critical pathogenic factor in Sjögren's syndrome. Targeting IL-12 and Tr1 cells may be a novel therapeutic strategy for the treatment of Sjögren's syndrome.

Disclosure of Interest: None declared

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SAT0017 THE RHEUMATOID FACTOR RESPONSE IS COMPOSED OF MULTIPLE REACTIVITIES AGAINST DIFFERENT EPITOPES

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Background: Rheumatoid arthritis (RA) is a complex autoimmune disorder in which autoantibodies likely play an important role. Rheumatoid factors (RFs) are autoantibodies that bind to the constant domain (Fc) of immunoglobulin G (IgG). It is known that they can bind in various ways; most RA patients probably have multiple types of RFs that all bind different parts of antibodies. It is presently unknown how this differs between patients. Furthermore, it is possible that RF responses in RA have a different reactivity pattern compared to those present in other diseases and in RF+healthy donors. Currently, these RA-specific RFs have not yet been identified.

Objectives: To develop a method to identify and dissect different RF responses, to classify the RF response in different stages of RA, and to characterise differences in reactivity between RF responses in the context of RA and RF responses in other diseases or healthy donors.

Methods: Variants of human IgG antibodies to which only a few different types of RFs can bind at a time were generated and used as target antibodies in newly

developed ELISA-based RF assays. RF responses were analysed in healthy donors, arthralgia patients at risk for RA and RA patients at different disease stages.

Results: RF responses in all cohorts were shown to be primarily directed against epitopes at the interface of the second and third IgG heavy chain domains (CH2-CH3) and the tip of the CH3 domain. The generated target antibodies were able to separate these RF responses and discriminate them from RF responses targeting epitopes outside these reactivity hotspots. Furthermore, certain RF responses against the CH2-CH3 interface were shown to depend on the presence of a single specific amino acid residue.

Pilot experiments using the target antibodies in clinical cohorts revealed that, in arthralgia patients and RA patients at various disease stages, the pattern of RF reactivity differs between individual patients as well as between cohorts. The degree to which an RF response against the CH2-CH3 interface depends on the single specific amino acid residue varied substantially between patients. One particular RF response pattern, RFs binding only the CH3 domain, was found exclusively in non-RA patients and healthy donors.

Conclusions: Using newly developed target IgGs, RF responses against different epitopes can be characterised. RF response patterns differ between RA and non-RA cohorts and between RA patients. These new tools may lead to identification of RA-specific RF responses and provide more insight into the pathogenic role of these autoantibodies.

Disclosure of Interest: None declared

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SAT0018

BLOOD CHEMOKINE SYSTEM PROFILE ASSOCIATED WITH DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

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Background: Chemokine receptors and their chemokine ligands (chemokine system), key mediators of inflammatory and immune cell trafficking, are involved in the pathogenesis of rheumatoid arthritis (RA). There is still limited information of blood chemokine system signature associated with disease activity.

Objectives: To identify RA-associated chemokine system signature using highly sensitive multiplex Proximity Extension ImmunoAssay (PEA) in serum together with analysis of CCR5, CCR6 and CXCR3 expression in blood cell subsets and assess its relationship with disease activity as evaluated by activity score (DAS28).

Methods: We investigated the serum levels of 92 inflammation-related proteins in 78 Czech patients with RA by PEA (Proseek Multiplex, Olink Bioscience, Sweden). Disease activity was assessed by means of DAS28 and subgroups were formed based on the disease activity, where DAS28 of ≥ 3.2 was taken as active RA (inactive RA, n=40; active RA, n=38). The expression of CCR5, CCR6 and CXCR3 receptors were analysed using 6-colour flow cytometry (BD FACSCanto II) on T and B lymphocytes, NK, dendritic cells, and monocytes in peripheral blood from 11 patients with active RA and 9 age-matched healthy control subjects. Statistical tests (Mann-Whitney U test, Spearman correlation) were performed using GenEx (Sweden) and GraphPad Prism 5.01 (CA, USA). *P*-value ≤ 0.05 was considered as significant.

Results: In patients with active RA, 16 serum proteins were upregulated (*P*_{corr} ≤ 0.05). Top-ranked proteins distinguishing active and inactive RA were CCL20, CXCL9, CCL7, CXCL10, IL-6, sCD137, and CXCL1 (*P*_{corr} < 0.03). Levels of all these proteins positively correlated with disease activity (*r* > 0.30 , *p* < 0.006) and the best correlation was observed for chemokines CCL20 (*r* = 0.495, *p* = 0.000004) and CCL7 (*r* = 0.442, *p* = 0.00005). Based on these results we analysed the expression of corresponding chemokine receptors: CCR5, CCR6, and CXCR3 in blood cells. Expression of CCR6 was increased on CD4⁺T and CD8⁺T lymphocytes (*p* = 0.008, *p* = 0.01 respectively) and on total and activated NK cells (*p* = 0.01, *p* = 0.004 respectively) in active RA compared with healthy controls. Regarding CCR5, decreased percentage of CCR5 positive CD4⁺T lymphocytes were registered in RA patients compared with healthy controls (*p* = 0.008). No differences were observed in the expression of CXCR3 on investigated immune cell populations.

Conclusions: We identified the blood chemokine system signature associated with disease activity in RA further supporting a critical role of CCL20/CCR6 axis in the ongoing inflammation related to the disease activity in RA.

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SAT0019

TNF- α REGULATES PLASMACYTOID DENDRITIC CELLS BY SUPPRESSING IFN- α PRODUCTION AND ENHANCING TH1 AND TH17 CELL DIFFERENTIATION

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Background: Human plasmacytoid dendritic cells (pDCs) play a vital role in modulating immune responses. pDCs can produce massive amounts of type I IFNs in response to nucleic acids via toll-like receptors (TLRs) and they are known to possess weak antigen-presenting properties inducing CD4⁺ T cell activation. Previous data showed a cross-regulation between TNF- α and IFN- α but the effect of TNF- α on pDCs remains unclear.

Objectives: To investigate how TNF- α regulates the immune function of human pDCs.

Methods: Fresh PBMCs were treated with TNF- α , TLR7 and TLR9 synthetic agonists. pDCs were immunophenotyped using flow cytometry. RNA from sorted pDCs was extracted and sequenced using Smart-seq2 for sensitive full-length transcriptome profiling. For pDC/T cell co-culture, fresh or TNF- α -treated pDCs were cultured with naïve CD4⁺ T cells for 5 days. The production of cytokines was measured by intracellular staining and ELISA.

Results: Upon stimulation with TLR7 and TLR9 agonists, there were three main pDC populations: non-producers, TNF- α -producers, TNF- α /IFN- α -producers. Exogenous TNF- α significantly reduced the production of both IFN- α and TNF- α in TLR9-stimulated pDCs but only IFN- α in TLR7-stimulated pDCs. Neutralisation of autologous TNF- α with anti-TNF antibody partially sustained IFN- α secretion by TLR9-stimulated pDCs after 24 hours. Exogenous TNF- α significantly promoted pDC maturation by upregulation of costimulatory molecules and chemokine receptors such as CD80, CD86, HLA-DR, and CCR7. RNAseq data analysis suggested that TNF- α inhibits IFN- α /TNF- α production by interfering with the NF- κ B pathway, promotes antigen processing and presentation pathways as well as T cell activation and differentiation. Indeed, TNF- α -treated pDCs induced *in vitro* higher CD4⁺ T cell proliferation and favoured Th1/Th17 polarisation.

Conclusions: Although pDCs possess weak antigen-presenting properties, TNF- α can enhance pDC maturation by switching their main role as IFN- α -producing cells to a more conventional DC phenotype. The functional status of pDCs might be strongly influenced by overall inflammatory environment and TNF- α might regulate IFN- α -mediated aspects of a range of autoimmune and inflammatory diseases.

Disclosure of Interest: None declared

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SAT0020

IMPACT OF IL12B SINGLE NUCLEOTIDE POLYMORPHISMS ON CIRCULATING PRO-INFLAMMATORY CYTOKINES IL-12P40 AND IL-23 IN ANKYLOSING SPONDYLITIS

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Background: Ankylosing spondylitis (AS) is an immune-mediated rheumatic disease belonging to the spectrum of axial spondyloarthritis, characterised mainly by chronic inflammatory back pain and radiographic sacroiliitis. Since genetic factors have been shown to be major determinants of susceptibility to the disease and contribute to amplification of immune responses, polymorphic variability in immune response genes are of interest. The interleukin-12p40 gene (IL12B) encodes the p40 polypeptide chain, which, together with p19, composes IL-23, a member of the IL-12 superfamily.

Objectives: In this study, we examined functional association between two IL12B polymorphisms – IL12B_{pro} (rs17860508) and IL12B 3' UTR A/C (rs3212227), and cytokine production of both pro-inflammatory cytokines IL-12p40 and IL-23 in AS patients.

Methods: A total of 69 AS patients with a mean age 43 $\pm$ 16 years and 257 healthy individuals from Bulgarian population were genotyped. Genotyping for the rs3212227 was performed by restriction fragment length polymorphisms-PCR assay and for the rs17860508 by allele specific-PCR. Serum IL-12p40 and IL-23 concentrations measurement was done using ELISA test.

Results: We found significant differences in the genotype (*p* = 0.029) and allele (*p* = 0.006) frequencies of rs17860508 polymorphism between AS patients and controls. An association between AS and the rs17860508 polymorphism was established under the allelic model (allele 2 vs. allele 1; OR = 1.698), the dominant model (1.2+2.2 vs. 1.1; OR = 2.427), and the co-dominant model (2.2 vs. 1.1;