components such as dsDNA or nucleosomes was shown to play an important role in breaking immune tolerance against nuclear antigens. Although the function of HMGB1, 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-denaturating detergent agarose gel electrophoresis (SDS–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 HMGB1c 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, these findings have significant physiological implications for the role of HMGB1 in cellular stress responses and innate immunity in lupus.

**REFERENCES:**


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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, FBS 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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**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. Expression of CCR6 was increased on CD4+ T and CD8+ T cells (p=0.000004) and CCL7 (r=0.442, p=0.00005). Based on these results we ana-

**Results:** The strongest correlation was observed for chemokines CCL20 (r=0.495, p<0.00001). Among all these proteins positively correlated with disease activity (r>0.30, p<0.006) was TNF-α (r=0.820, p<0.00001). Top-ranked proteins distinguishing active and inactive RA were TNF-α (p=0.0000007), IL-12p70 (p=0.000009), and CCL7 (r=0.442, p=0.00005). Based on these results we ana-

**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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**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 spondyloarthritides, 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 is of interest. The interleukin-12p40 gene (IL12B) encodes the p40 polypeptide chain, which, together with p19, comprises IL-23, a member of the IL-12 superfamily.

**Objectives:** In this study, we examined functional association between two IL12B polymorphisms – IL12Bpro (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±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;