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