DENDRITIC CELL-DERIVED IL-27 REGULATES THE MAGNITUDE OF INDUCIBLE ECTOPTIC GERMINAL CENTRES IN SALIVARY GLANDS, FAILING TO MODULATE IL-17 PRODUCTION IN CD4 T CELLS FROM PATIENTS WITH SJÖGREN’S SYNDROME

Davide Lucchesi1, Rachel Coleby1, Elena Pantorina1, Edoardo Prediletto1, David Hill2, Alicia Derrac Soria2, Felice Rivelles3, Costantino Pitzalis1, Simon Jones4, Gareth Jones4, Michele Bombardieri5, William Harvey Research Institute, Queen Mary University of London, Experimental Medicine and Rheumatology, London, United Kingdom; 2Cardiff University, School of Medicine, Cardiff, United Kingdom; 3University of Bristol, School of Cellular and Molecular Medicine, Bristol, United Kingdom

Background: Approximately 30% of Sjögren’s Syndrome (SS) patients develop Eccotrop Lympohid Structures (ELS) in their salivary glands (SG), ELS play an active role in autoimmunity and contribute to the development of MALT lymphoma. Interleukin 27 (IL-27) exerts key immunomodulatory actions on CD4 T cells with both pro and anti-inflammatory roles but its role in the formation and regulation of ELS in the salivary glands of SS is unknown.

Objectives: We first used a murine model of inducible SG EL27 to elucidate the role of IL-27 and its interaction with IL-17 in the regulation of ELS formation and function. We then extended our observations on a cohort of SS patients to identify IL-27 cellular source, target cells and functional properties in modulating peripheral and lesional CD4 T cell function.

Methods: To trigger ELS formation a single dose of reporter-encoding adenovirus was delivered directly to the SG of wild-type (WT) and IL-27RA-deficient (KO) mice. For IL-17 blockade anti-mouse IL17A antibody was administered systemically. ELS development and peripheral immune responses were tracked by immune-histopathology, FACS, and qPCR. Minor SG biopsies were collected from SS and non-specific sialadenitis (sicca) patients. Peripheral blood mononuclear cells (PBMCs), isolated CD4 T cells and parotid gland MCs were incubated with IL-27 and analysed by FACS for CD4 T cell subsets while cytokine levels were measured intracellularly by FACS and in culture supernatants. Tissue IL-27 was assessed in SS SG sections by multicolour immunofluorescence to identify IL-27 producing cells.

Results: In WT mice, SG ELS formation was preceded by an upregulation of IL-27 and infiltration of IL-27 producing cells (CD11b+ first followed by CD4 and CD8 T cells). KO mice displayed larger, more abundant ELS in the SG with more germinal centres and higher levels of ELS-related genes (Cxcl13, Ccl19, Ltb, Aid) compared to WT mice. During ELS formation, KO mice had an uncontrolled SG Th17 response and systemic IL-17A blockade caused reduction in ELS size and in the expression of ELS-related genes. In SS patients SG and serum, we observed higher expression levels of IL-27 transcripts and protein, respectively, compared to sicca, while SG IL-27 was selectively increased in the ELS+ subset of SS. Immuno-fluorescence staining for IL-27 revealed its presence primarily in the T cell rich areas of SG ELS with frequent co-localization with DC-LAMP+ dendritic cells. Finally, while IL-27 was able to significantly downregulate IL-17 production in HD, CD4 T cells from patients with SS failed to downregulate IL-17 but showed an aberrant IFNγ release upon IL-27 incubation. We do not observe any difference in the PBO expression or downstream STAT1/3 phosphorylation between SS and HD.

Conclusion: IL-27 is a critical regulator of the magnitude of the germinal centre response in the SG by restricting Th17 expansion. Both in murine inducible ELS and in vitro experiments PBMCs, isolated CD4 T cells and parotid gland MCs were incubated with IL-27 and analysed by FACS for CD4 T cell subsets while cytokine levels were measured intracellularly by FACS and in culture supernatants. Tissue IL-27 was assessed in SS SG sections by multicolour immunofluorescence to identify IL-27 producing cells.

Disclosure of Interests: Davide Lucchesi: None declared, Rachel Coleby: None declared, Elena Pantorina: None declared, Edoardo Prediletto: None declared, David Hill: None declared; Alicia Derrac Soria: None declared, Felice Rivelles: None declared, Costantino Pitzalis Grant/research support from: Celgene, Simon Jones: None declared, Gareth Jones: None declared, Michele Bombardieri Grant/research support from: Celgene, Consultant for: Medimmune DOI: 10.1136/annrheumdis-2019-eular.5889

DNA SEQUENCING AND MACHINE LEARNING TECHNIQUES PREDICT MAJOR ORGAN INVOLVEMENT IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

Anastasia Filia1, George Bertasi2, Nikolaos Panouzis1, Dionissi Nikolopoulos1, Emmanouil Dermitsakis2, Dimitrios Boumpas1,1 Biomediical Research Foundation Academy of Athens, Athens, Greece; 2University of Crete, Medical School, Heraklion, Greece; 3University of Geneva, Medical School, Geneva, Switzerland; 4National and Kapodistrian University of Athens, Athens, Greece

Background: Both clinically and molecularly, Systemic Lupus Erythematosus (SLE) is a heterogeneous disease with non-synchronous multi-organ involvement of varying severity, and alternating periods of remission and flares. There is an unmet need for a blood-based “liquid biopsy” to predict prognosis of the disease.

Objectives: To define the smallest set of genes predicting SLE organ involvement and disease activity using RNA-sequencing data derived from whole blood cells from 150 SLE patients and machine learning techniques.

Methods: Disease activity was measured by the SLE disease activity index-2000 (SLEDAI-2K) and by organ involvement (treated as binary outcomes). SLEDAI-2K: mild-moderate disease (SLEDAI 0-8), severe (SLEDAI >8). Organ involvement: major organ (renal, heart, lung, nervous system and SLEDAI-2K ≥10) and minor (all others). The RNA-sequencing dataset was pre-processed to assemble 20,368 genes and then split in training/validation data. Two feature selection steps (edgeR and recursive feature elimination) were used to remove noise and keep the smallest set of genes which best predicts each outcome. Different prediction models were fit to identify which one performs best using the gene signature selected in the previous step.

Results: Two gene signatures were kept after feature selection to predict each of the two outcomes (25 genes for organ involvement; 50 genes for SLEDAI-2K). Organ involvement was predicted with high accuracy (accuracy=0.89, sensitivity=0.85, specificity=0.88 in the validation data) using the elastic net generalised linear model. Among the 25 best predictors were MPO, ITDQ4 and CD38. SLEDAI-2K could not be predicted with high accuracy using the neural net model. Performance was still the same even when 1648 genes (after first feature selection step) were used as predictors of SLEDAI-2K. The performance of these models will also be tested in an independent test dataset once available (currently undergoing sequencing).

Conclusion: The model predicting organ involvement performed better compared to the model predicting SLEDAI-2K. This could be attributed to the fact that certain disease manifestations are not currently included in SLEDAI-2K. Further analysis and functional laboratory experiments of those genes will help to identify biomarkers for more accurate assessment of disease activity and prognosis in the clinic.

Acknowledgement: This work was supported by FOREUM, SYSCID and ERC-Advanced Grant.


BIOMARKER PROFILING REVEALS NOVEL MECHANISTIC INSIGHTS INTO USTEKINUMAB THERAPEUTIC RESPONSES IN SYSTEMIC LUPUS ERYTHEMATOSUS

Matteo Cesaroni1, Looqman Seridi1, Jarat Jordan1, Kristen Sweet1, MA Keying1, Carol Franklin1, Jessica Schreiber1, Peter Lipsky1, Ronald van Vollenhoven2, Bevara H. Hahn3, Shawn Rose1, Frederic Baribaud1, Matthew J Loza1, Kim Campbell1, George Tkos3, Janssen Research and Development, LLC, Spring House, United States of America; 2AMPel, Biosolutions, LLC, Charlottesville, United States of America; 3Amsterdam Rheumatology and Immunology Center, Amsterdam, Netherlands; 4University of California, Los Angeles, Los Angeles, United States of America; 1Both internal Darmouth-Maclean Medical Center, Harvard Medical School, Boston, United States of America

Background: Systemic lupus erythematosus (SLE) is a heterogenous autoimmune disease that causes progressive organ damage. The cytokine type I interferon (IFN-I), IL-12 and IL-23 have all been shown to contribute to SLE pathogenesis. We previously reported that treatment with ustekinumab (UST), an anti-IL-12/23 p40 neutralizing monoclonal antibody, improved global and organ-specific measures of disease activity in a randomized, placebo (PBO)-controlled study of patients with active SLE (NCT02349801). Here, we utilized biomarker data from this clinical study to elucidate the mechanism of action of UST in SLE.

Objectives: We aimed to determine whether modulation of IL-12, IL-23, or both cytokines was associated with clinical efficacy, and to ascertain whether UST treatment could modulate IFN-I or improve disease activity in patients exhibiting an elevated IFN-I signature at baseline.

Methods: A Phase 2, placebo (PBO)-controlled study enrolled 102 patients with seropositive SLE and active disease despite standard-of-care therapy. Patients were randomized 3:2 to receive UST IV ~6 mg/kg or PBO at week 0, then subcutaneously every 8 weeks. Whole blood RNA from PAXgene tubes and serum were collected over 24 weeks. Age and sex-matched healthy controls were also studied. Serum IFN-I, IL-17A, IL-17F and IL-22 levels were quantified by ELISA and an indicative of the IFN-I pathway (IFN-I) and the IFN-γ pathway (IFN-γ). ELISA was utilized to quantify the IFN-I pathway. Whole blood RNA was assayed for gene expression by microarray. Two Th172,3,4 an IFN-γ5 gene signature and 21-gene IFN-I signature (IGS) was analyzed. SLE Responder Index (SRI)-4 at week 24 was used to define UST response (UST-R) and non-response (UST-NR).

Results: Serum IL-17A, IL-17F and IL-22 levels and Th17 signature levels in blood remained largely stable over the course of 24 weeks in all treatment groups. In contrast, UST-R was associated with a durable reduction in IFN-γ protein and IFN-γ gene signature levels relative to baseline, which was not observed

Disclosure of Interests: None declared

in UST-NR or PBO patients. IGS levels were elevated in 67% of patients at baseline versus healthy controls. Serum IFN-γ levels and IGS levels in blood were not modulated by UST treatment through week 24. Baseline IFN-I signature status did not associate with response to UST, as the treatment effect size (UST vs PBO) was similar in IGS low (Δ=27%) and high (Δ=28%) patients.

Conclusion: Response to UST was associated with reductions in IFN-γ levels, whereas IL-17A, IL-17F, IL-22 and IFN-I remained largely unchanged. While these findings require confirmation in an ongoing Phase 3 study, these data imply involvement of the IL-12 pathway and suggest a novel mechanism of action for UST-R in SLE.

REFERENCES:

Disclosure of Interests: Matteo Cesaroni Employee of: Janssen Research & Development, LLC, Lomeane Seridi Employee of: Janssen Research & Development, LLC, Jarrat Jordan Employee of: Janssen Research & Development, LLC, Kristen Sweet Employee of: Janssen Research & Development, LLC, Keying Ma Employee of: Janssen Research & Development, LLC, Carol Frank Employee of: Janssen Research & Development, LLC, Jessica Schreiter Employee of: Janssen Research & Development, LLC, Peter Lispky Consultant for: Consulting fees from Horizon Pharma, Ronald van Vollenhoven Grant/research support from: AbbVie, AstraZeneca, Biotest, Bristol-Myers Squibb, Celgene, Crescendo, GlaxoSmithKline, Janssen, Merck, Merck, Novartis, Pfizer, Roche, UCB, Vertex, Bevra H. Hahn Grant/research support from: Janssen Research & Development, LLC, ShawnRose Employee of: Janssen Research & Development, LLC, Frederic Barbibaud Consultant for: Johnson & Johnson, Elisa Corsiero: None declared, Francesca Barone Consultant for: GlaxoSmithKline, Roche, UCB Pharma, Actelion, ONO Pharmaceutical, Consultant for: GlaxoSmithKline, Roche, Actelion, ONO Pharmaceutical, Benjamin Fisher Consultant for: Novartis, Roche, MedImmune, Frédéric Baribaud Shareholder of: Johnson & Johnson, Roberto Giacomelli Consultant for: Actelion, BMS, Lilly, Sobi, Edoardo Prediletto: None declared, Elisa Corsiero: None declared, Francesca Barone Grant/research support from: GlaxoSmithKline, Roche, UCB Pharma, Actelion, ONO Pharmaceutical, Consultant for: GlaxoSmithKline, Roche, Actelion, ONO Pharmaceutical, Benjamin Fisher Consultant for: Novartis, Roche, MedImmune, Bristol-Myers Squibb, Serena Colafrancesco: None declared, Ilaria Puxeddu: None declared, Roberto Giacomelli Grant/research support from: Pfizer, Speakers bureau: MSD, BMS, Lilly, Sobi, Edoardo Prediletto: None declared, Elisa Corsiero: None declared, Francesca Barone Grant/research support from: GlaxoSmithKline, Roche, UCB Pharma, Actelion, ONO Pharmaceutical, Consultant for: GlaxoSmithKline, Roche, Actelion, ONO Pharmaceutical, Benjamin Fisher Consultant for: Novartis, Roche, MedImmune, Bristol-Myers Squibb, Serena Colafrancesco: None declared, Ilaria Puxeddu: None declared, Roberto Giacomelli Grant/research support from: Pfizer, Speakers bureau: Actelion, Bristol-Myers Squibb, Merck Sharp & Dohme, Abbvie, Pfizer, Sobi, Sobi, Roberto Priori: None declared, Chiara Baldini: None declared, Costantino Pititzis Grant/research support from: Celgene, Michele Bombardi Grant/research support from: Celgene, Consultant for: MedImmune


RESULTS: IL21 and IL21R expression, together with CD4+CD45RO+PD1+ICOS+ Tfh cells were strongly enriched in ELS+ vs ELS- SS SGs. Tfh cells densely infiltrated B cell rich areas and, within ectopic GC, acquired BCL6 expression, both in ELS+ and MALT-L. Tfh infiltration significantly correlated with SG IL21 mRNA levels, which in turn was associated with high T (CD3+), B (CD20+) and plasma cells (CD138+) IHC scores and significantly correlated with CXCCL3, LTj, BAFF, AID and Pax5 gene expression. MALT-L samples displayed 10-fold higher IL21 mRNA and twice as much PD1+ICOS+BCL6+ Tfh-cells field in comparison to ELS+ SS samples. Within the SGs, IL21 and INFγ were the most abundant cytokines produced by infiltrating CD4+ T cells and CXCR5+ Tfh cells frequently displayed double IL21/INFγ production. Similarly, IL21, IL17 and INFγ producing ICOS+CXCR5+ circulating Tfh cells were significantly increased in the peripheral blood of SS patients compared to sicca, but only IL21+ Tfh cells were associated with the presence of ectopic GC in SG. IL21, IL17 and INFγ production by Tfh in the peripheral compartment showed a positive correlation with IgG serum levels.

Conclusion: Within the SG of SS patients Tfh cells closely segregate with lesional IL21 expression, localize within ELS and are strongly enriched during MALT-L development, which is supporting their role in sustaining B cell activation and malignant transformation. High production of IL21 by Tfh in peripheral blood selectively identifies SS patient with ectopic GC in the SG, suggesting a role for IL21+ Tfh cells as surrogate markers of SS histopathology in SS.

Acknowledgement: This work was supported by project grants from the Medical Research Council (MR/N003863/1 to MB) and Versus Arthritis (grant 21753 to EP).

Disclosure of Interests: Elena Pontarini: None declared, William Murray Brown: None declared, Cristina Croia: None declared, Davide Lucchesi: None declared, Felice Rivellese: None declared, Liliane Fossati-Jimack: None declared, Piero Ruscitti: None declared, Eduardo Huarte, Paul Smith, Monika Scorron: Incyte Corporation, Wilmington, United States of America

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease that can affect multiple organs, including the skin, joints and kidneys. The disease is characterized by the development of skin lesions, autoantibody production and inflammation-induced glomerulonephritis. The skin is involved in up to 85% of SLE cases. In the MRL/lpr spontaneous mouse model of SLE, disease symptoms also include lymphocytic hyper-proliferation, resulting in lymphadenopathy and splenomegaly. The evolutionary conserved Janus kinase-signal transducer of activators of transcription (JAK-STAT) pathway constitutes a rapid membrane to nuclear signaling module that affects key biological aspects of the mammalian immune system. Janus kinase 1 (JAK1) is involved in the downstream signaling pathway of type I interferons (IFNs), and high levels of type I IFNs are associated with SLE. INCBO54707 is an oral small molecule JAK1 selective inhibitor currently being evaluated in clinical trials for the dermatological disease hidradenitis suppurativa (NCT03569371 & NCT03807487). We hypothesized that INCBO54707 should reduce IFN signaling and ameliorate cutaneous lesions in SLE.

Objectives: To determine the in vivo effectiveness of INCBO54707, a selective JAK1 inhibitor, in a preclinical model of cutaneous lupus erythematosus.

Methods: Female MRL/lpr mice were randomized to treatment groups at 11 weeks old to receive twice daily oral doses of vehicle (0.5% methyl cellulose) or INCBO54707 at 10, 30, or 90 mg/kg for 10 weeks. Efficacy was determined by weekly scoring of the changes in skin, lymph node size, and proteinuria. At study termination (week 21), inguinal lymph nodes and spleens were excised and weighed, skin lesions and kidneys were excised and fixed for histopathologic analysis, and serum was collected for pharmacokinetic analysis and ELISA. Autoantibody presence in the serum was detected using a commercial anti-dsDNA ELISA kit.

Results: In vitro enzymatic selectivity screening of INCBO54707 revealed JAK1 IC50 = 8.9 nM versus JAK2 IC50 = 463 nM, resulting in a JAK1/JAK2 selectivity


THE SELECTIVE JAK1 INHIBITOR INCBO54707 AMELIORATES CUTANEOUS LESIONS IN A SPONTANEOUS MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATEOUS

Brittany Fay, Xin He, Alexander Margulis, Yan-Ou Yang, Yu Li, Wenqing Yao, Eduardo Huarte, Paul Smith, Monika Scorron. Incyte Corporation, Wilmington, United States of America

Background: Systemic lupus erythematosus (SLE) is an autoimmune disease that can affect multiple organs, including the skin, joints and kidneys. The disease is characterized by the development of skin lesions, autoantibody production and inflammation-induced glomerulonephritis. The skin is involved in up to 85% of SLE cases. In the MRL/lpr spontaneous mouse model of SLE, disease symptoms also include lymphocytic hyper-proliferation, resulting in lymphadenopathy and splenomegaly. The evolutionary conserved Janus kinase-signal transducer of activators of transcription (JAK-STAT) pathway constitutes a rapid membrane to nuclear signaling module that affects key biological aspects of the mammalian immune system. Janus kinase 1 (JAK1) is involved in the downstream signaling pathway of type I interferons (IFNs), and high levels of type I IFNs are associated with SLE. INCBO54707 is an oral small molecule JAK1 selective inhibitor currently being evaluated in clinical trials for the dermatological disease hidradenitis suppurativa (NCT03569371 & NCT03807487). We hypothesized that INCBO54707 should reduce IFN signaling and ameliorate cutaneous lesions in SLE.

Objectives: To determine the in vivo effectiveness of INCBO54707, a selective JAK1 inhibitor, in a preclinical model of cutaneous lupus erythematosus.

Methods: Female MRL/lpr mice were randomized to treatment groups at 11 weeks old to receive twice daily oral doses of vehicle (0.5% methyl cellulose) or INCBO54707 at 10, 30, or 90 mg/kg for 10 weeks. Efficacy was determined by weekly scoring of the changes in skin, lymph node size, and proteinuria. At study termination (week 21), inguinal lymph nodes and spleens were excised and weighed, skin lesions and kidneys were excised and fixed for histopathologic analysis, and serum was collected for pharmacokinetic analysis and ELISA. Autoantibody presence in the serum was detected using a commercial anti-dsDNA ELISA kit.

Results: In vitro enzymatic selectivity screening of INCBO54707 revealed JAK1 IC50 = 8.9 nM versus JAK2 IC50 = 463 nM, resulting in a JAK1/JAK2 selectivity