OP0276

DENDRITIC CELL-DERIVED IL-27 REGULATES THE MAGNITUDE OF INDUCIBLE ECTOPIC GERMINAL CENTRES BUT FAILS TO DOWNMODULATE IL-17 PRODUCTION IN CD4 T CELLS FROM PATIENTS WITH SJÖGREN'S SYNDROME

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Background: Approximately 30% of Sjögren's Syndrome (SS) patients develop Ectopic Lymphoid 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 ELS 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 cells 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 IL-17A antibody was administered systemically. ELS development and peripheral immune responses were tracked by immuno-histopathology, FACS, and qPCR. Minor SG biopsies were collected from SS and non-specific sialadenitis (sicca) patients. Peripheral blood mononuclear cells (PBMC) isolated from patients and age/sex matched healthy donors (HD). For 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 cytokines 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-27p28 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. Immunofluorescence 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 did not observe any difference in IL-27R 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 patients with SS, dendritic cells appear as the main cellular source of IL-27. Although SG IL-27 was increased in the ELS+ subset of SS, it consistently failed to downregulate IL-17 release in CD4 T cells from SS patients suggesting that a profound dysregulation of the IL-27/IL-17 axis play an important role in ELS formation in this condition.
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OP0277

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

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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 detect 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 $\geq$8). Organ involvement: major (renal, heart, lung, central nervous system & SLEDAI-2K$\geq$6) 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=0.89, specificity=0.88 in the validation data) using the elastic net generalised linear model. Among the 25 best predictors were *MPO, ITGA3* and *CD38*. SLEDAI-2K could not be predicted with high accuracy (accuracy 0.75, sensitivity=0.79, specificity=0.67) using the neural network 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.
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OP0278

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

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Background: Systemic lupus erythematosus (SLE) is a heterogenous autoimmune disease that causes progressive organ damage. The cytokines 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 (NCT02349061).[1] 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.[1]Patients were randomized 3:2 to receive UST IV ~6 mg/kg or PBO at week 0, then subcutaneous injections of 90mg UST q8w or PBO. Whole blood RNA from PAXgene tubes and serum were collected over 24 weeks. Age and sex-matched healthy controls were also studied. Serum IFN-γ, and IL-17A, IL-17F and IL-22 levels were quantified by ELISA as indicative of the IL-12 and IL-23 pathways, respectively, and an IFN-α ELISA was utilized to quantify the IFN-I pathway. Whole blood RNA was assessed for gene expression by microarray. Two Th17[2,3], an IFN-γ[4] gene signature and 21-gene IFN-I signature (IGS)[5] were 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 gene 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

in UST-NR or PBO patients. IGS levels were elevated in 67% of patients at baseline versus healthy controls. Serum IFN-α 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 implicate the involvement of the IL-12 pathway and suggest a novel mechanism of action for UST-R in SLE.

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OP0279

A UNIQUE IL-21 SIGNATURE CHARACTERIZES LESIONAL AND CIRCULATING T-FOLLICULAR HELPER CELLS IN SJÖGREN'S SYNDROME PATIENTS WITH ECTOPIC GERMINAL CENTRES AND MALT LYMPHOMA

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Background: B/T cell aggregates in the salivary glands (SG) of Sjögren's syndrome (SS) can form ectopic lymphoid structures (ELS) with germinal centers (GC), which are linked to MALT lymphoma (MALT-L) development. T follicular-helper cells (Tfh), main producers of IL21, are crucial for affinity maturation and class-switching of GC B cells, and may be responsible for onset of autoimmunity.
Objectives: To characterize the cytokine production in circulating T cells alongside the Tfh infiltration and the expression of IL21/IL21 receptor (IL21R) in the SGs of SS patients, in the context of ectopic GCs and MALT-L.
Methods: SG biopsies with matching histology and RNA from 37 SS and 38 non-specific chronic sialadenitis (NSCS) patients were stratified as ELS-/ELS+ based on CD3/CD20/CD21/CD138 immunostaining (IHC). Histological samples and mRNA from 12 parotid MALT-L were also analyzed. Gene expression was measured by Taqman RT-PCR. Multicolor immunofluorescence/confocal microscopy for CD3, CD4, CD45RO, ICOS, PD1, BCL6 identify Tfh cells in SG and MALT-L tissues. FACS analysis for CD4, CD8, INFγ, IL17, IL21, Granzyme-B, IL10, CD25, Foxp3, CXCR5, ICOS, PD1, was performed on peripheral blood mononuclear cells from 20 SS and 10 NSCS patients with matched SG histology for the characterization of T CD4+ cells cytokines production.
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 level, which in turn was associated with high T (CD3+), B (CD20+) and plasma cells (CD138+) IHC scores and significantly correlated with CXCL13, LTβ, 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 IFNγ 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 IFNγ 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 SG histopathology in SS.
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OP0280

THE SELECTIVE JAK1 INHIBITOR INCB054707 AMELIORATES CUTANEOUS LESIONS IN A SPONTANEOUS MURINE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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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[1]. In the MRL/lpr spontaneous mouse model of SLE, disease symptoms also include lymphocyte 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 nucleus signaling modality that affects key biological aspects of the mammalian immune system[2]. 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[3]. INCB054707 is an oral small molecule JAK1 selective inhibitor currently being evaluated in clinical trials for the dermatological disease hidradenitis suppurativa (NCT03569371 & NCT03607487). We hypothesized that INCB054707 should reduce IFN signaling and ameliorate cutaneous lesions in SLE.
Objectives: To determine the effectiveness of INCB054707, 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 INCB054707 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 INCB054707 revealed JAK1 IC50 = 8.9 nM versus JAK2 IC50 = 463 nM, resulting in a JAK1/JAK2 selectivity