IFNα stimulation.[1 3] Notably, autophagy has been implicated in the secretion of inflammatory cytokines (so-called secretory autophagy),[2] however, its possible involvement in BAFF production remains unexplored. We herein sought to delineate the role of secretory autophagy in BAFF secretion by SLE and IFNα-stimulated CD14+ monocytes.

Methods To evaluate unconventional BAFF secretion from IFNα- stimulated CD14+ monocytes we administered Brefeldin A (ER-Golgi transport inhibitor) in vitro and BAFF, IL1β and TNFα secretion were measured by ELISA. BAFF expression was also assessed by flow cytometry. The contribution of secretory autophagy was examined, in IFNα/SLE sera – exposed CD14+ monocytes, by gene silencing studies (for Atg5/Rab8a/Rab11) and by ex vivo treatment with Bafilomycin A1, 3MA and GW4869 (autophagy and exosome release inhibitors). In a translational approach, we isolated SLE patient monocytes and measured BAFF expression both at mRNA (RT- PCR) and protein levels (ELISA). The potential BAFF-LC3B-CD63 co localization was evaluated by immunofluorescence.

Results IFNα-stimulated CD14+ monocyte cell cultures in the presence of brefeldin A, (conventional secretory pathway inhibitor), failed to inhibit BAFF secretion; instead, interference with the autophagy secretory machinery through silencing of *Atg5*, *Rab8a*/*Rab11*, or the pharmacological inhibitors Bafilomycin, 3MA and GW4869, resulted in significant reduction of BAFF secretion. By contrast, induction of autophagy by starvation or mTOR inhibition (rapamycin) correlated with increased BAFF production. Importantly, IFNα stimulated and active SLE CD14+ monocytes produced copious amounts of BAFF and exhibited significant colocalization of BAFF with the autophagy marker LC3B and the exosome marker CD63 (figure 1).

Conclusions Our data provide strong evidence that, under the effect of IFNα, BAFF secretion by monocytes engages in an autophagy/exosome- based secretory pathway. Pending experimental procedures corroborating the presence of BAFF within the exosomes will strengthen our working hypothesis. Delineation of the BAFF secretory pathway may pave the way for novel targeted treatments in SLE.

Acknowledgement The research work is supported by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 6729).

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P18 PROTEOMIC ANALYSIS OF CUTANEOUS LUPUS ERYTHEMATOSUS (CLE) DERMAL INFILTRATES AND CONTROL SKIN REVEALS DIFFERENTIALLY UPREGULATED AND DOWNREGULATED PATHWAYS

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10.1136/lupus-2024-el.72

Background Many investigators have explored pathways upregulated in SLE and CLE. Few reports studied key downregulated pathways and mediators. Using unbiased proteomic technique, we have previously identified IL-16 as a key cytokine in cutaneous lupus erythematosus (CLE), and findings were verified lupus nephritis.[1 2] In this analysis we analysed the downregulated pathways in the same proteomic database.

Objectives To systematically identify patterns of protein expression in dermal infiltrates of skin lesions of patients with CLE in comparison to control skin, and explore what pathways might be downregulated or non-functional.

Methods Skin biopsies from 6 CLE patients and 6 controls were investigated as described before.[2] Inflammatory infiltrates and control dermis were laser capture micro-dissected and run on nano-LC tandem mass spectrometry.

Data was analysed by String and Qiagen Ingenuity pathway analysis (IPA). P-values<0.05 were considered significant, adjustment for multiple testing was performed.

Results Comparing CLE vs controls we identified 300 proteins that were upregulated in CLE, while 387 were downregulated.

The IPA of the upregulated pathways was presented before,[2] and include interferon, EIF2, hyper-cytokinemia, mitochondrial dysfunction, RHOGDI signalling, Th1 and Th2, granzyme A pathways.

There were a high number of downregulated pathways, which included remodelling of epithelial adherent junctions, fatty acid a-oxidation, Sertoli-cell junction, GP6, apelin liver, wound healing, and a list of downregulated degradation pathways for tryptophan X, putrescine, dopamine, valine, histamine, noradrenaline and adrenaline, ethanol; followed by integrin and epithelial adherent junction signaling, oxidative phosphorylation, estrogen receptor and intrinsic phrothrombin activation. Overlapping among several pathways, components of respiratory chain (NADH dehydrogenase and multiple subcomponents), galectin 3 and 7, epidermal growth factor receptor (EGFR), interleukin-1 receptor antagonist protein (IL1RN) and integrin alpha 2 (ITGA2) were significantly

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downregulated, while for example ITGA beta 2 (ITGAB2) was strongly upregulated.[2]

Conclusions Comparative analysis of upregulated and downregulated pathways at the site of skin inflammation, have disclosed several key-components that are downregulated, possibly malfunctioning or dysbalanced in regulation of inflammation and tissue regeneration. This novel information may contribute to better understanding of disease molecular pathogenesis, and what pathways might be of interest for pharmaceutical targeting.

Acknowledgements Karolinska Institutet-Mayo Clinic collaboration fund, NCI Cancer Center Support Grant 5P30 CA15083–43C1, Signe och Reinhold Sunds fund and Margareta Nilsson foundation.

Disclosures Timothy Niewold: None declared; Alexander Meves: None declared; Julia Lehman: None declared; Karin Popovic-Silwerfeldt: None declared; Cristine Charlesworth: None declared; Benjamin Madden: None declared; Elisabet Svenungsson shareholder of: AstraZeneca, Pfizer, Speakers fee: Janssen, Grant/research support from: Merck; Vilija Oke Speakers fee: Novartis, Jansen, Astra Zeneca, UCB, Eli Lilly and Abbvie, Grant/research support from: Ono pharma.

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P19 REGULATION OF MONOCYTE INTRACELLULAR METABOLISM IN THE INFLAMMATORY ENVIRONMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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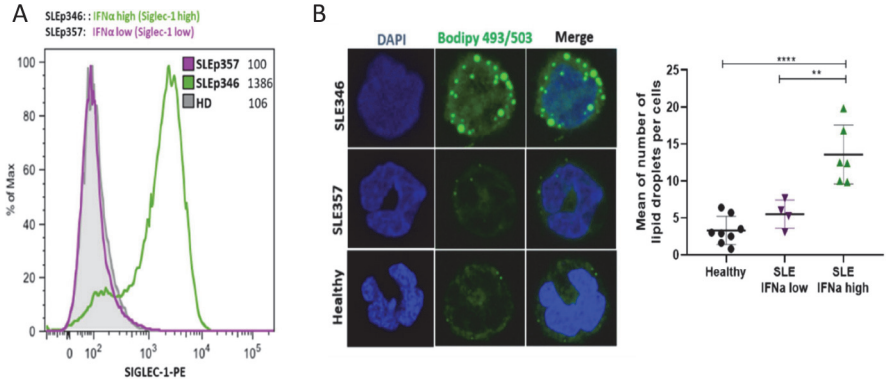
10.1136/lupus-2024-el.73

Objective In SLE, monocytes are instructed by type I interferon (IFNα) to be activated and produce inflammatory mediators. Metabolome studies in SLE patient sera have revealed distinct perturbations of lipid metabolism.[1 2] However, the metabolic alterations of SLE monocytes under the effect of IFNα and their contribution to disease pathogenesis remain ill-defined. Our aim is to delineate the metabolic repercussion of monocytes in response to prolonged IFNα signaling in the context of SLE.

Methods RNA was extracted from $CD14^+$ monocytes of active SLE patients and healthy donors and the latter were cultured in the presence or absence of recombinant IFNα. RNA sequencing was performed followed by differential gene expression and Gene Set Enrichment Analysis (GSEA). $CD14^+$ monocytes were isolated from the abovementioned groups and cholesterol levels and lipid droplet formation were assessed by colorimetric assays and confocal microscopy. Cytokine release and antigen presenting capacity of IFNα-stimulated monocytes, in the presence or absence of metabolic inhibitors, were evaluated by ELISA and flow cytometry, respectively.

Results Transcriptome analysis in IFNα-treated CD14+ monocytes revealed significant enrichment of inflammation and lipid-related genes, which are implicated in cholesterol homeostasis. These molecular aberrations displayed significant overlap with differentially expressed genes (DEGs) from SLE $CD14^+$ monocytes with $IFN\alpha^{high}$ gene signature as compared to their counterparts with $IFN\alpha^{low}$ gene signature. The direct effects of IFNα on cholesterol synthesis were confirmed by the increased intracellular cholesterol levels and enhanced lipid droplet formation both in IFNα-stimulated monocytes and in SLE monocytes with $IFN\alpha^{high}$-signature (figure 1). Notably, the blockade of cholesterol biosynthesis was able to reduce the IFNα-mediated inflammatory phenotype of monocytes as evidenced by the reduced secretion of IL-6 and TNF-α, as well as reduced expression of the co-stimulatory molecules CD86 and CD40 (figure 2).

Conclusions Our data suggest that IFNα-mediated perturbations in lipid-related pathways may contribute to the development of SLE inflammation. These results may offer a mechanistic explanation for the increased disease burden within $IFN\alpha^{high}$ SLE patients.



Abstract P19 Figure 1 (A) The levels of SIGLEC-1 were assessed by monitoring its expression using flow cytometry (n=3). **(B)** A representative confocal image of immunofluorescence microscopy depicting lipid droplets (green: BODIPY 493/503) is shown. The bar plot shows the number of lipid droplets per donor Healthy donors n=8, SLE patients n=10. Results are shown as mean ± SEM; *p < 0.05; **p < 0.01; ***p < 0.001.

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