underwent demographic data collection, sCAL assessment (ELISA Buhlmann sCAL diagnostic test), and disease activity assessment by SLEDAI-2K for SLE patients. Patients were divided into two groups, active disease and remission, taking SLEDAI-2K value of 6 as a cut-off, and defined patients who had recent vascular incidents associated with SLE.

Results 13 patients in active group (38.5% women, median age 50 (35–58), SLE duration 8.85±6.26 years, SLEDAI-2K 14 (7–21)) were compared to 14 patients in remission group (85,7% women, median age 39 (35–53), disease duration 5.21 ±2.78 years, SLEDAK-2K 3.5 (2–4)) and to 13 control subjects (76,9% women, median age 25 (24–26)). Significantly higher occurrence of neuropsychiatric disease (53.8% vs. 14.3%, P=0.032) and vascular manifestations (76.9% vs. 14.3%, P=0.001) was observed in active patients compared to those in remission. We haven't observed the difference in occurrence of APS, serositis, dermatologic, constitutional, renal, or hematologic manifestations of SLE between groups. sCAL levels were higher in all patients compared to the control group (1.1 (0.53–1.8) µg/ml vs. 0.7 (0.38–1.1) µg/ml, P=0.02). Moreover, sCAL was higher in the active group compared to remission patients (1.6 (1.1–3.0) µg/ml vs. 0.8 (0.2–1.4) µg/ml, P= 0.02). Patients with a history of recent vascular events had greater sCAL respect other SLE patients (1.7 (1.15–3) µg/ml vs. 1.0 (0.225–1.375) µg/ml, P=0.015). There was a positive correlation between SLEDAI and sCAL in all patients (ρ=0.532, P=0.0043), particularly in active ones (ρ=0.673, P=0.0017). There was no correlation between SLEDAI and sCAL within inactive patients (ρ=0.0678, P=0.818).

Conclusions SLE patients, especially those with active disease, had higher sCAL compared to healthy subjects. We established a positive correlation between vascular manifestations of SLE and sCAL, but also between SLEDAI-2K and sCAL in patients with active disease. Our study was limited due to the small sample size. Further research should confirm these hypotheses on a larger number of subjects.

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P17 AUTOPHAGY- BASED UNCONVENTIONAL SECRETION OF B CELL ACTIVATING FACTOR (BAFF) BY MONOCYTES IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE)

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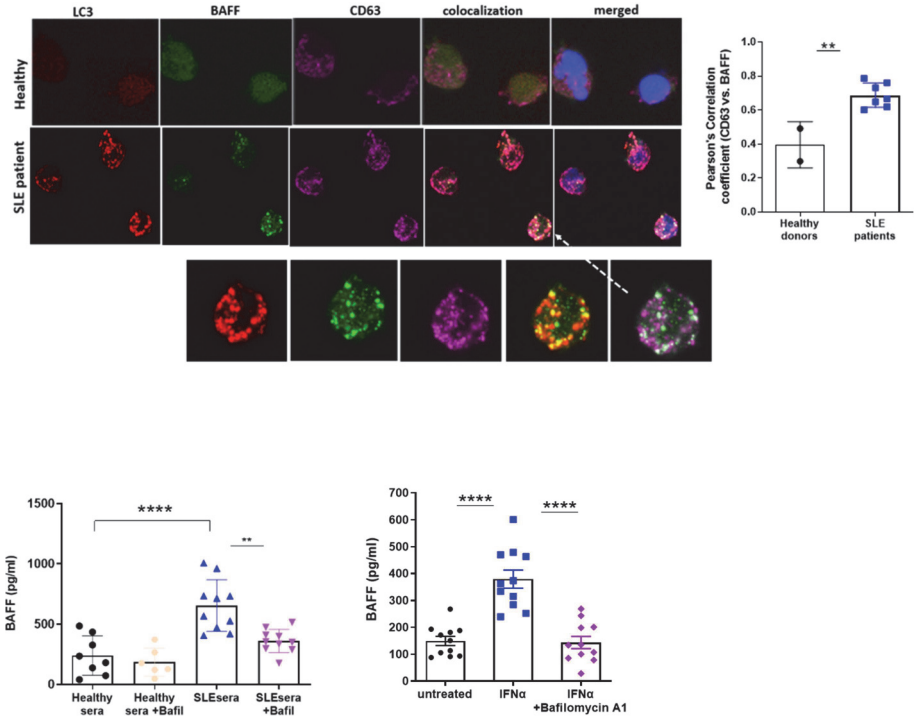
Objective CD14+ monocytes, play pivotal role, in SLE pathogenesis through enhanced production of B-cell activating factor (BAFF), a major cytokine driving B-cell maturation into autoantibodies-secreting plasma cells. Lupus monocytes demonstrate deregulated autophagy and inflammatory phenotype upon



Abstract P17 Figure 1 Autophagy -dependent secretion of BAFF in SLE patient-derived or IFNα stimulated CD14+ monocytes (a) Representative confocal image of immunofluorescence microscopy of anti-LC3A/B, anti- CD63 and anti- BAFF (green: BAFF, red: LC3A/B, magenta: CD63) blue: 4′,6-diamidino-2-phenylindole (DAPI)/DNA) are shown. Scale bars: 10µm. Pearson's co-localization coefficient for BAFF CD63 and LC3A/B. Each dot represents an independent SLE donor from 100 cells each. (n=7) and bar plots show the mean ± SEM expression. ****P < 0.0001 (two-tailed, Mann-Whitney test). (b) BAFF secreted levels by CD14+ monocytes cultured with 20% SEL active sera +/- Bafilomycin or stimulated with IFNα (c).

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.

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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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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