Methods We developed a murine monoclonal antibody to tissue bound C3d (mAb 3d29) that targets tissue-bound C3d when injected in vivo. We stained biopsy tissue from patients with active LN to confirm that 3d29 detects tissue C3d in human disease. We also analyzed biopsy reports from 76 patients with LN to assess whether C3 deposits correlate with disease activity.

We next radiolabeled mAb 3d29 with ^{124}I and injected 16-week-old MRL/lpr mice (a model of lupus-like disease) or wild-type mice intravenously with 20 μg of protein (120 μCi). The mice underwent whole body PET imaging using a Siemens Inveon microPET/CT scanner at 4, 24, 48, 72, and 96 hours post injection. After the mice were sacrificed, organs were weighed and counted in a gamma counter along with a known amount of the radioactive injectate to determine the biodistribution of the antibody.

Results By immunostaining, mAb 3d29 detects glomerular C3d in kidney samples from MRL/lpr mice and humans with LN. In patients with LN, the abundance of glomerular C3 fragments (0-3+) was highest in those patients with proliferative disease and with high activity scores.

^{124}I-PET with mAb 3d29 revealed rapid tracer uptake in the kidneys of MRL/lpr mice in the first hours after injection compared to controls, with retention up to 96 hours (figure 1). At the end of the study (144 hours), there was still a trend towards greater antibody in kidneys of MRL/lpr mice than controls (4.93±1.42% vs 1.77±0.17 percentage of injected radioactivity per gram of tissue, P=0.07)

Conclusion Glomerular C3 deposition is an important marker of disease activity in LN. C3d-imaging can be used to non-invasively detect inflammation in the kidneys of patients with LN.

509 THE LOCALIZATION OF NOVEL MACROPHAGE SUBSETS IN CLASS III AND IV LUPUS NEPHRITIS KIDNEY SECTIONS

[1,2]Paul J Hoover*, [1]Michael Peters, [3]Jeff Hodgin, [1]Tony Jones, [1]Jon Chen, [4]Anne Davidson, [1]Nir Hacohen. *[1]Broad Institute of MIT and Harvard, Cambridge MA, USA; [2]Division of Rheumatology, Immunology, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston MA, USA; [3]Department of Pathology, Michigan Medicine, University of Michigan, USA; [4]Feinstein Institutes for Medical Research, Manhasset NY, USA*

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Background Macrophage infiltration in lupus nephritis is associated with fibrosis and kidney damage. Prior histologic studies lacked the specificity of single-cell RNA sequencing (scRNA-seq) for macrophage classification, so it was impossible to determine how the spatial organization of each subset related to kidney remodeling. Our recent scRNA-seq of macrophages has defined 3 novel subsets enriched in lupus nephritis over healthy kidneys: 'inflammatory' macrophages likely enter lupus kidneys from blood and shift to 'phagocytic' and 'reparative-like' states. Here, we mapped their positions in kidney sections from 20 different lupus nephritis patients using subset-specific transcripts from our scRNA-seq data to reveal new macrophage spatial phenotypes.

Methods We collected and sectioned archived FFPE class III or IV index lupus nephritis kidney biopsies from Brigham and Women's Hospital. After standard FFPE antigen retrieval, we used commercial RNA probes against 2-3 highly specific genes based on scRNA-seq to stain each novel subset (inflammatory CSF1R+/CD300E+/CD36-, phagocytic CSF1R+/CD300E+/CD36+; reparative CSF1R+/RNASE1+), and probes against non-mammalian genes as a negative control. We identified our macrophage subsets based on the presence of probes above the background and within 3 microns of the DAPI-stained nucleus as a cell boundary estimate. For spatial mapping, we transferred annotated histologic features from an adjacent H&E section to sections stained for cells. For cell distance and density measurements we used HALO (Indica Labs).

Results Our *in situ* staining approach confirmed the presence of the novel inflammatory, phagocytic, and reparative macrophages discovered by scRNA-seq in class III and IV lupus nephritis kidney sections. Most inflammatory and phagocytic macrophages were localized to positions inside glomeruli while a smaller proportion in the tubulointerstitium formed a gradient toward the glomerular borders. Reparative macrophages were the most abundant macrophage subset *in situ* and were mostly in the tubulointerstitium arranged as a gradient toward glomerular borders. The abundance of reparative macrophages inside glomeruli varied across patients.

Conclusions Macrophage subsets were spatially localized to and around the glomerulus in lupus nephritis kidney sections. Inflammatory and phagocytic macrophage subsets were mostly inside the glomerulus, suggesting that glomerular factors supported their recruitment from blood and *in situ* differentiation. The most abundant subset - reparative macrophages - were localized to the tubulointerstitial space and arranged in a gradient toward glomerular borders, indicating a chemical attraction to nephritic glomeruli and the presence of factors that promote reparative differentiation. Interestingly, the abundance of reparative macrophages inside glomeruli varied considerably across patients, raising the possibility that interpatient variability reflects differences in kidney function that we are now testing in an expanded cohort.

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510 B-CELL INTERFERON-β CORRELATES WITH LUPUS NEPHRITIS IN SYSTEMIC LUPUS ERYTHEMATOSUS

[1,2,3]Fatima Alduraibi, [4]Huma Fatima, [5]Jennie A Hamilton, [1]Walter Winn Chatham*, [1]Hui-Chen Hsu, [1,2]John D Mountz. *[1]Department of Medicine, the University of Alabama at Birmingham, Birmingham, AL, USA; [2]Birmingham VA Medical Center, Birmingham, AL, USA; [3]Department of Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, SA; [4]Department of Pathology, the University of Alabama at Birmingham, Birmingham, AL, USA; [5]Department of Medicine, the University of Tennessee at Memphis, Memphis, TN, USA*

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Background Early diagnosis of lupus nephritis (LN) can be challenging since some patients do not exhibit overt clinical manifestations until advanced stages. B cell interferon-beta (IFNβ) correlates with development of B cell autoreactive phenotype. The objective of the present study is to determine if elevated IFNβ in circulating B cells can be a useful indicator for the development of more severe histopathologic features of LN.

Methods Flow cytometry was used to quantitate intracellular IFNβ in naive (IgD+CD27−) CD19+ B-cells in the peripheral blood mononuclear cells (PBMCs) of a cross-sectional cohort (N=80) of patients with systemic lupus erythematosus (SLE), 33 of whom had lupus nephritis. Serologic and clinical manifestations of LN included anti-DNA, anti-Sm, C3, C4, and urine protein/creatinine ratio were determined. The correlation of B-cell IFNβ with lupus nephritis classification and

histopathological findings, light, electron microscopy, and immunofluorescence (IF) for deposition of IgM, IgG, IgA, C1q, and C3 was determined in 23 of the 33 patients for whom renal biopsy data was available.

Results LN was identified in 41% of our cohort of 80 SLE patients. Naïve B-cell IFNβ was positively associated with the development of LN but not cutaneous disease. Higher levels of B-cell IFNβ also correlated with higher levels of circulating anti-dsDNA, anti-Sm, and the urinary protein/creatinine ratio. Biopsy examination revealed that proliferative LN lesions (Class III, IV with or without V) characterized by significantly elevated endocapillary hypercellularity, fibrous crescent, and fibrocellular crescent were significantly associated with high B-cell IFNβ. Surprisingly, IgG, IgA, IgM, C3, and C1q deposition in the kidney was not correlated with B-cell IFNβ.

Conclusions Our results suggest that B-cell IFNβ can be used in combination with other clinical diagnostic markers to assist in identifying patients who are at high risk of developing advanced LN.

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511 DISEASE FLARES IN LUPUS ARE CONCORDANT WITH *RUMINOCOCCUS BLAUTIA GNAVUS* BLOOMS ARISING WITHIN UNSTABLE GUT MICROBIOTA COMMUNITIES

[1]Doua F Azzouz, [1]Ze Chen, [1]Peter Izmirly, [2]David Fenyo, [1]Jill Buyon, [3]Alexander V Alekseyenko, [1]Gregg J Silverman*. *[1]Department of Medicine, NYU Grossman School of Medicine, New York; [2]Institute for Systems Genetics, NYU Grossman School of Medicine, New York; [3]Biomedical Informatics Center, MUSC Charleston, 29203*

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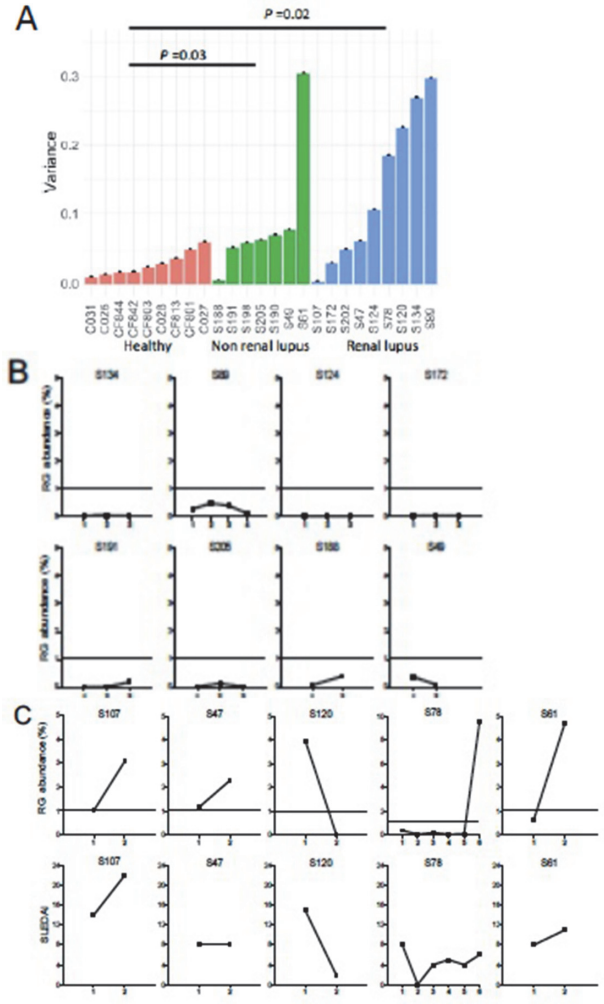
Background Whereas genetic susceptibility for the development of the archetypic autoimmune disease, Systemic Lupus Erythematosus, has been well explored the precipitants for clinical disease flares remain a mystery. We have therefore investigated for dynamic time-dependent relationships between gut-microbial communities and Lupus disease activity.

Methods Patients fullfilled ACR criteria and were not treated with recent antibiotics or cytotoxic agents. Disease activity defined by modified SLEDAI, with fecal 16S rRNA libraries generated, and data were analyzed as previously described. Taxonomic surveys were performed on sequential 16S rRNA gene amplicon-libraries from 16 individual female Lupus patients and 22 healthy volunteers from fecal samples obtained at serial timepoints, over many months to several years.

Results Lupus patients commonly displayed significant imbalances in alpha and beta microbiota-diversity, and patients were uniquely different from the healthy individuals as well as different from other Lupus patients – a pattern of disease-associated heterogeneity in dysbiotic communities termed *the Anna Karenina Principle*. From community-wide ecological multivariate analysis of sequential libraries obtained overtime, patients displayed significantly greater longitudinal microbiota community instability that was most exaggerated in Lupus Nephritis patients. Furthermore, taxonomic analyses documented an absence of bacterial intestinal blooms in 9 healthy adults with up to 12 serial fecal 16S libraries. In contrast, many of the microbiota communities of 16 Lupus patients demonstrated transient spikes of pathogenic bacterial species, with by far the most prevalent were blooms of *Ruminococcus blautia gnavus* (RG), which were documented in 4/9 Lupus Nephritis and 1/7 non-renal patients, concordant with disease flares. Importantly,



Abstract 511 Figure 1 Lupus patients have more unstable gut microbiota than healthy individuals, and a subset of Lupus patients have blooms of *Ruminococcus blautia gnavus* concordant with disease flares. (A) To compare the overall dynamics of shifts in fecal communities sampled overtime in different subjects, subject variances were computed based on Jensen-Shannon Divergence using the Tw2 statistic. Variances in these three groups were significantly different (Kruskal Wallis ANOVA, $p = 0.03$). Patients with Lupus nephritis, based on ACR criteria, whereas the patients in the non-renal group were without a history of documented lupus nephritis. Temporal-dependent variance documents instability in the gut microbiota communities of Lupus patients compared to healthy. (B) In 11 SLE patients, a stable low abundance of RG representation was detected. (C) In 5/16 (31%) of SLE patients evaluated overtime, abundance of RG fluctuated greatly overtime. In these cases, RG abundance at much higher levels were present in fecal samples obtained proximal to visits in which disease flares were documented. In all but one excluded of these patients had documented LN. RG relative abundance was evaluated for 16 SLE in 44 samples obtained at different time points, and for CTL subjects in 49 samples obtained at different time points, which ranged from 2-12 samples per donor. Dotted line depicts 1% threshold of 16S rRNA amplicon representing RG abundance. Note that in panel C, for patient S78 the greater range of RG abundance necessitated a different scale. SLEDAI $\geq$ 8 was considered high disease activity.