Results B cells grouped into 4 clusters: GCs (DZ and LZ), plasma cells and memory B cells. DZ, LZ and PC clusters were represented in similar proportions in both conditions; whereas, MemB cells were more expanded in the immunized chimeras. Using the paired single cell BCR sequences and repertoire analysis, we observed clones with clear expansion both in the autoimmune and immunized chimeras. We observed levels of mutation in a similar range though DZ, LZ and MemB from autoimmune mice had a significantly higher number of nucleotide replacement mutations, and the reverse was observed in PCs. Nevertheless, PCs in both conditions reached similar maximum levels of mutation. Interestingly, autoimmune cells showed more isotype diversification in all compartments. Notably, we observed distinct gene expression for the autoreactive B cells such as CXCL10 by GC B cells and SLPI expression by autoreactive plasma cells. Strikingly, we identified DN2- and DN4-like memory B cells in both conditions.

Conclusions We find WT B cells break tolerance, expand in GC and develop into MemB and PCs in a seemingly unrestricted manner, similar to immune mice. Results should open the way to new approaches to control pathogenicity of rogue B cells in autoimmune disease.

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1703 ACTIVATED PI3Kδ SIGNALS COMPROMISE PLASMA CELL SURVIVAL VIA LIMITING AUTOPHAGY AND INCREASING ENDOPLASMIC RETICULUM STRESS

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Background Understanding key signals that control the differentiation, function, and survival of plasma cells (PCs) is critical for development of improved therapeutic approaches to attenuate pathogenic antibody responses in SLE. While phosphatidylinositide 3-kinase delta (PI3Kδ) plays an essential role in humoral immune responses, its role(s) in PC function remains poorly understood.

Methods We utilized a conditional mouse model of Activated PI3Kδ Syndrome (APDS), to interrogate the role of this key signaling program.

Results Mice expressing a gain-of-function mutation in *PIK3CD* in B cells, referred to as activated (a) PIK3CD, generated increased numbers of memory B cells, mounted enhanced secondary response, yet exhibited a rapid decay of antibody levels over time. Consistent with these findings, aPIK3CD expression markedly impaired plasma cell generation. Remarkably, PC specific aPIK3CD expression was sufficient to diminish humoral responses in vivo. Mechanistically, aPIK3CD disrupted endoplasmic reticulum proteostasis and autophagy, leading to increased PC death. Notably, this defect was driven primarily by elevated mTORC1 signaling and modulated by treatment with PI3Kδ-specific inhibitors.

Conclusions Taken together, these data demonstrate an unexpected requirement to down-regulate PI3Kδ activity to balance autophagy and the unfolded protein response, events essential to modulate ER stress and ensure PC survival. Thus, enhancing PI3Kδ activity may provide a novel means to trigger early PC death and dampen autoantibody responses.

1704 IDENTIFYING CLUSTERS OF LONGITUDINAL AUTOANTIBODY PROFILES ASSOCIATED WITH SYSTEMIC LUPUS ERYTHEMATOSUS DISEASE OUTCOMES

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Background Prior studies of SLE clusters based on autoantibodies have utilized cross-sectional data from single centers. We applied clustering techniques to longitudinal and comprehensive autoantibody data from a large multinational, multiethnic inception cohort of well characterized SLE patients to identify clusters associated with disease outcomes.

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Methods We used demographic, clinical, and serological data at enrolment and follow-up visits years 3 and 5 from 805 patients who fulfilled the 1997 Updated ACR SLE criteria and were enrolled within 15 months of diagnosis. For each visit, ANA, dsDNA, Sm, U1-RNP, SSA/Ro60, SSB/La, Ro52/TRIM21, histones, ribosomal P, Jo-1, centromere B, PCNA, anti-DFS70, lupus anticoagulant (LAC), IgG and IgM for anti-cardiolipin, anti–β2GP1, and aPS/PT, and IgG anti-β2GP1 D1 were performed at a single lab (except LAC). K-means clustering algorithm on principal component analysis (10 dimensions) transformed longitudinal ANA/autoantibody profiles was used. We compared cluster demographic/clinical outcomes, including longitudinal disease activity (total and adjusted mean SLEDAI-2K), SLICC/ACR damage index and organ-specific domains, SLE therapies, and survival, using one-way ANOVA test and a Benjamini-Hochberg correction with false discovery rate alpha=0.05. Results were visualized using t-distributed stochastic neighbor embedding.

Results Four unique patient clusters were identified (table 1). Cluster 1, characterized by high frequency of anti-Sm and anti-RNP over time, was the youngest group at disease onset with a high proportion of subjects of Asian and African ancestry. At year 5, they had the highest disease activity, were more likely to have active hematologic and mucocutaneous involvement, and to be on/exposed to immunosuppressants/biologics. Cluster 2, the largest cluster, had low frequency of anti-dsDNA, were oldest at disease onset, and at year 5, had the lowest disease activity, and were least likely to have nephritis and be on/exposed to immunosuppressants/biologics. Cluster 3 had the highest frequency of antiphospholipid antibodies over time, were more likely to be of European ancestry, have an elevated BMI, be former smokers, and by year 5, to have nephritis, neuropsychiatric involvement, including strokes and seizures (SLICC/ACR damage index). Cluster 4 was characterized by anti-SSA/Ro60, SSB/La, Ro52/TRIM21, histone antibodies, and low complements at year 5. Overall, survival of the 805 subjects was 94% at 5 years, and none of the clusters predicted survival.

Conclusions Four SLE patient clusters associated with disease activity, organ involvement, and treatment were identified in

Abstract 1704 Table 1 Demographic and clinical characteristics that were statistically significant[1] at baseline and five-year follow-up between the four SLE longitudinal autoantibody clusters

	Group 1 (n=137)	Group 2 (n=377)	Group 3 (n=79)	Group 4 (n=212)	p-value	FDR
Enrolment Demographics						
Mean Age of Diagnosis (SD), yrs	32.0 (10.8)	36.9 (13.9)	32.9 (14.0)	34.9 (14.0)	0.003	0.003
% Ethnicity						
White	32	62	68	43	<0.001	<0.001
Asian	31	20	14	31	0.001	0.004
African	27	10	6	15	<0.001	<0.001
Mean BMI (SD), kg/m^2	25.5 (6.1)	26.4 (6.6)	26.9 (6.9)	24.9 (5.4)	0.015	0.041
% Former smoker	17	23	28	12	0.002	0.005
Clinical Characteristics at Year 5 Follow-Up						
% Nephritis[2]	42	30	46	42	0.028	0.009
Mean SLEDAI-2K Score (SD)						
Total Score[3]	4.3 (4.5)	2.3 (3.3)	3.0 (3.1)	3.6 (3.2)	<0.001	<0.001
Adjusted Mean Score[4]	4.3 (3.1)	2.9 (2.5)	3.7 (2.2)	4.1 (2.6)	<0.001	<0.001
Hematological Subscale	0.16 (0.39)	0.06 (0.23)	0.06 (0.25)	0.11 (0.31)	0.003	0.008
Immunological Subscale	1.7 (1.69)	0.82 (1.30)	1.82 (1.54)	2.12 (1.62)	<0.001	<0.001
Low Complement	0.82 (0.99)	0.43 (0.82)	1.01 (1.01)	1.03 (1.00)	<0.001	<0.001
Mucocutaneous Subscale	0.83 (1.98)	0.33 (0.87)	0.20 (0.69)	0.37 (0.97)	<0.001	<0.001
Mean SLICC Damage Index (SD)						
Neuropsychiatric Domain	0.11 (0.40)	0.16 (0.52)	0.32 (0.82)	0.08 (0.32)	0.003	0.009
Cerebrovascular accident	0.02 (0.15)	0.04 (0.22)	0.14 (0.45)	0.04 (0.19)	0.002	0.007
Seizures	0.01 (0.12)	0.03 (0.16)	0.08 (0.27)	<0.01(0.07)	0.004	0.012
Skin Domain	0.15 (0.42)	0.07 (0.29)	0.03 (0.16)	0.07 (0.28)	0.011	0.030
Alopecia	0.11 (0.31)	0.04 (0.20)	0.01 (0.11)	0.04 (0.19)	0.002	0.007
Medications Ever						
% Immunosuppressives/Biologics	79	60	61	72	<0.001	<0.001
% Imuran	51	31	41	41	<0.001	<0.001
% Mycophenolic Acid	34	21	18	25	0.014	0.039
Medications Current						
% Immunosuppressives/Biologics	62	45	48	55	0.004	0.012

1. Comparison between cluster groups using one-way ANOVA test and a Benjamini-Hochberg correction with false discovery rate (FDR) alpha = 0.05
2. Lupus nephritis was diagnosed by renal biopsy or fulfillment of the renal item on the ACR classification criteria.
3. The total score of SLEDAI-2K is the sum of all 24 descriptor scores. The total SLEDAI-2K score falls between 0 and 105, with higher scores representing higher disease activity.
4. A measurement of lupus disease activity over time determined by the calculation of the area under the curve of SLEDAI-2K over time by adding the area of each of the blocks of visit interval and then dividing by the length of time for the whole period.

Abbreviations: BMI, body mass index; SD, standard deviation; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; SLICC, Systemic Lupus International Collaborating Clinics; yrs, years.

this analysis of longitudinal ANA/autoantibody profiles in relation to SLE outcomes, suggesting these subsets might be identifiable based on extended autoantibody profiles early in disease and carry prognostic information.

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1705 PROTEIN ASSEMBLAGES ARE NEWLY DESCRIBED INTRACELLULAR STRUCTURES THAT MAY PLAY A ROLE IN SHAPING THE LUPUS AUTOANTIBODY REPERTOIRE

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Background Why are autoantibodies in systemic autoimmunity directed against only ~5% of the proteome? It has recently been discovered that many intracellular proteins, rather than being evenly distributed throughout the cell, instead form assemblages (also known as Membraneless Organelles and Biological Condensates) that arise from phase separation of their protein components, akin to partitioning of oil droplets in water. Such a conformation might be potentially more immunogenic than that of proteins with a diffuse presence in cells. We wondered if lupus autoantigens might preferentially exist as assemblages and thereby provoke autoantibody production.

Methods We obtained from an assemblage prediction algorithm (Vernon et al., elife 7, 2018) the propensity scores (PScores), i.e., likelihood, for phase separation of autoantigens and non-autoantigens. We then compared autoantigens with the highest PScores to identify shared structural properties. We used the European Molecular Biology Laboratory 'InTact' Molecular Interactions Database to assess the potential for interactions of autoantigens compared with non-autoantigens.

Results The mean PScores for autoantigens (n = 1050) and the entire human proteome of non-autoantigens (n = 17,532) were 1.46 and 1.09 (p = 1.2E- 08). To varying extents, the 25 autoantigens with the highest phase separation propensities shared additional features such as compositional bias, repeated domains, coiled coil regions, nucleic acid binding, and disorder. Most of these properties were present with greater frequencies than observed for non-autoantigens. When potential interactions were compared using InTact, autoantigens had at least a ten-fold greater tendency to interact with themselves and other proteins.

Conclusions We suggest that assemblage formation and certain protein structural features are key factors in determining the spectrum of lupus autoantibodies. Assemblages may promote autoantibody formation by concentrating certain intracellular proteins, as concentrations may be ~100x or more that of the intracellular proteins not contained in assemblages or organelles. Assemblages also foster protein-protein interactions, which could lead to changes in conformation of one or both partners, resulting in novel conformations to which the immune system might not be tolerized.

1706 A MODEL OF LUPUS PATHOGENESIS: ANTI-EBNA1 HETEROANTIBODIES INITIATE LUPUS BY CROSS REACTING WITH LUPUS AUTOANTIGENS, RESULTING IN LUPUS AUTOANTIBODIES AND CLINICAL DISEASE

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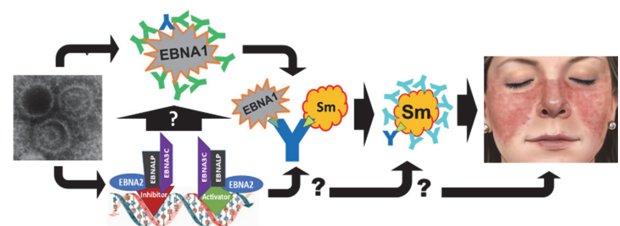
Background Mechanisms explaining the well-known association of SLE with Epstein-Barr virus (EBV) infection are unknown. In other experiments, the Epstein-Barr nuclear antigen-2, -3C, & -LP (EBNA2, EBNA3C, EBNALP), all EBV-encoded transcription co-factors, have been shown to concentrate at SLE genetic risk loci, supporting the hypothesis that the host immune response to EBV initiates SLE autoimmune processes. Cross-reactions of the anti-EBNA1 heteroimmune response with the Sm B/B', Sm D, Ro/SSA, and C1q autoantigens are convincing. Some of these cross reacting EBNA1 epitopes appear to be the initiating the autoantibodies in SLE patients and induce SLE-like disease in animals after immunization. EBNA1 aberrantly reduces T cell responses in normal individuals. Perhaps, the first SLE autoantibodies usually emerge from the anti-EBNA1 humoral response. If this hypothesis is the common sequence of events in SLE, then anti-EBNA1 responses would be predicted to be more frequent in EBV-infected SLE patients than in EBV-infected controls.

Methods We tested this prediction using the large dataset in East Asians published by Cui, et al (*PLoS One* 2018;13: e01931711) by matching controls to cases by age and sex and using conditional logistic regression.

Results All 232 SLE patients (100%) were EBV-infected, while 54 of 696 patient controls were not EBV infected (7.6%), resulting in a strong association (OR=28.6 (6.4-∞, p=5x10^{-8}), further confirming the known close association of EBV infection with SLE with a 100% attributable fraction. Most importantly, virtually all the SLE cases tested for both anti-VCA IgG and anti-EBNA1 IgG also had anti-EBNA1 antibodies (124 of 125 (99.2%)), which were more frequent than in age- and sex-matched controls (232 of 250 (93.2%)) (OR=9.7, 95%CI 1.5-414, p=0.0078) for an 89.7% additional attributable fraction among those EBV-infected, thereby adding anti-EBNA1 antibodies as an SLE risk factor beyond EBV infection.



Abstract 1706 Figure 1

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