patients. There was a significant correlation of the percent of IL4R-/-iNOS-/- naïve B cells with SLEDAI, anti-Sm and anti-DNA. In vivo pre-treatment of Bx22 mice with IL-4 significantly blocked R848 induction of CD11c^+CD21^- Tbet^- DN2 B cells in the spleen. This was associated with a significant decrease in anti-DNA, anti-histone, anti-Sm, and anti-RNP autoantibodies. scRNA-seq analysis revealed that the molecular mechanism for IL-4 suppression of the R848 response was mediated through an transcriptome of aerobic metabolism and an IL-4-induced 1 (IL4i1)-Aryl hydrocarbon receptor (AhR) pathway. IL-4 inhibition of DN2 B cell development in human B cells in vitro was partially inhibited by the AhR inhibitor CH-223191.

Conclusion Low expression of the IL-4R program and low signaling through IL-4R at the Tr and naïve B cell stages in SLE pre-disposes such B cells to activation through TLR7. This can upregulate signaling through type II interferon and along with other stimuli to promote development of pathogenic DN2 B cells. Development of DN2 B cells can also be inhibited by IL-4R pathway agonist including treatment of cells with IL4i1 or other major activators of the AhR or tryptophan metabolites, such as IAA and IALD. Further studies of these pathways and molecules that can effectively and beneficially modify them could lead to improved treatment for SLE.

Lay ABSTRACT SLE is associated with excessive activation of lymphocytes, primarily B lymphocytes that can produce autoantibodies and pathogenic cytokines. Excessive B cell activation in SLE can be linked to several activation molecules including type I interferon, type II interferon, and cytokines including IL-17 and others. Much of the focus for treatment of SLE has been on neutralizing the pathogenic cytokines that drive the development of pathogenic B cells. In contrast, very little attention has been directed towards understanding how B cells in SLE patients become susceptible to activation through diverse pathways, and if lowered levels of immune mediators that maintain B lymphocyte in a quiescent status can contribute to the initiation of the autoimmune process. Previous studies are shown that while most of the inflammatory cytokines and factors are upregulated in SLE, IL-4 and its receptor (IL4R) are consistently downregulated in SLE patients. The present results have studied the effects of the low expression of IL-4R in the initial stages of B cell development in SLE. We have identified that IL-4R inhibited low expression whereas type I interferon exhibited high expression in SLE patients compared to normal controls. This imbalance in IL-4 and type I interferon resulted in a differential gene program regulation of B cells that prompts the activation and development of B cells into a pathogenic “DN2” population of B cells. In contrast, in normal controls, this IL4R-programmed naïve B cell development promotes a different B-cell developmental trajectory that do not result in production of autoantibodies. The effects of IL-4 inhibiting pathogenic B cell development was analyzed in humans and in a mouse model of lupus. In both cases, pre-treatment with IL-4 blocked TLR7 and type I interferon-induced pathogenic “DN2” B cell development and suppressed the circulating levels of autoantibodies that are commonly seen in SLE patients. The molecular mechanism for IL-4R suppression of development of pathogenic B cells in lupus was found to be based on key molecules that regulate metabolism and quiescence of B cells. Further studies of these pathways and molecules that can effectively and beneficially modify them could lead to improved treatment for SLE.

B cells

LONGITUDINAL IMMUNE CHANGES DURING AND AFTER RECENT FLARES IN LUPUS

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Background Previous studies suggest substantial immunologic heterogeneity in lupus. However, the majority of these studies were cross-sectional in nature. Here we followed flaring and quiescent patients longitudinally to determine how their immunologic profile changes over time.

Methods Forty-seven SLE patients with a recent flare (change in clinical SLEDAI ≥ 2 in the past month that prompted a change in therapy), 25 quiescent SLE patients (clinical SLEDAI = 0 for ≥ 1 year with no increase in immunosuppressive treatment , ≤ 10 mg prednisone, matched for disease duration) and 16 healthy controls (HC) were recruited. The peripheral blood immunologic profile at baseline and follow-up (every 6 months for 1 year, COVID permitting) was examined by multi-parameter flow cytometry. Expression of interferon (IFN)-induced proteins that correlated with gene expression was examined in immune populations of interest using CyTOF.

Results Using unsupervised clustering, incorporating all subjects and visits, four distinct immunologic profiles were seen: Cluster 1, with increased levels of activated B cells and age-associated B cells (ABCs); Cluster 2, with Thh and Thp expansion; Cluster 3, with reduced levels of innate, naïve B, and Thh cells; and Cluster 4 with expansion of Th1 and innate immune cells relative to other clusters. Although patients with new-onset flares were found in all clusters, Cluster 1 had the highest number of these patients, whereas Cluster 4 has the highest number of patients who were inactive at baseline, as well as HC. Patients moved between clusters over time and/or in response to treatment. A substantial proportion of flaring patients in Cluster 3 transitioned to Cluster 1 on follow-up, suggesting that B cell changes accumulate post-flare. Similar findings were seen for myeloid populations in a smaller subset of patients that transitioned from Cluster 3 to 4. In general, patients in Cluster 1, 2, or 4 at baseline tended to remain in the same cluster subsequently, with a notable exception being patients with early disease (< 6 months duration), where switching between clusters was frequent. Patients in Cluster 1 at follow-up were more likely to remain active or flare than those in Cluster 4. Analysis of IFN-induced protein expression, revealed considerable variability in the levels of these proteins between immune populations in the same patient and between patients, with significantly higher levels in flaring than in quiescent patients in most immune populations. Cluster 1 visits tended to have higher levels of IFN-induced proteins than Cluster 4 visits, particularly within B cell populations and the T helper cell populations that support their activation.

Conclusion Accumulation of activated B cells and ABCs can occur during or after flare, is associated with high levels of IFN-induced proteins in these populations, and defines
patients who are more likely to have ongoing disease activity or subsequent flares.

206 INFLAMMATION CRITICALLY REGULATES ANTIBODY PRODUCTION
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10.1136/lupus-2022-lupus21century.10

Antibodies are generated through two distinct pathways following B cell activation: the rapid generation of short-lived antibody-secreting plasma blasts and plasma cells (so called extrafollicular responses, EFRs) and the slower development of germinal center reactions (GCs). During the latter, B cells undergo clonal expansion, diversification and affinity maturation, and the generation of long-lived plasma cells as well as memory B cells that often respond to a repeat challenge with EFRs. The mechanisms regulating the differentiation of B cells along one or the other response type are incompletely understood, but critical for understanding how to intervene in ineffective or harmful humoral responses. Using a mouse model of influenza infection we demonstrate that the development of EFRs is dependent on B cell intrinsic and extrinsic, inflammatory, Toll-like receptor (TLR) signals. B cell-intrinsic TLR signals supported antigen-stimulated B cell survival, clonal expansion, and the differentiation of B cells via induction of IRF4, the master regulator of B cell differentiation, through activation of NF-kB c-Rel. Provision of sustained TLR4 stimulation after immunization altered the fate of virus-specific B cells towards EFRs instead of GCs. Thus, acute inflammatory signals enhance antibody production as a means to provide rapidly protective antibodies in infections. During chronic inflammation, the same signals may drive a continued and potentially harmful autoantibody response, indicating that control of inflammation may curb pathogenic antibody responses.

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B Cell Biology

207 ABC AND EXTRAFOLCULAR RESPONSES: MECHANISTIC INSIGHTS FROM MURINE MODELS OF LUPUS AND INFECTION
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Background Animal models and more recently studies in humans have implicated the “extrafollicular” (EF) B cell response—rather than the germinal center (GC) response—as being prominent and possibly pathogenic in lupus. This response can also generate so-called “age- associated B cells” (ABC, known also as “DN2” in humans) under some conditions, a type of inflammatory B cell the frequency of which is elevated in some lupus patients and animal models of lupus. However, the basic biology that underlies why some immune responses are directed toward the EF mode and others to the GC mode is not well-understood. Nor are the identities and functions of ABC well-defined, particularly in lupus. ABC have been termed memory B cells, implying a quiescent or resting state, which seems incompatible with an ongoing inflammatory disease like lupus and raising the question of how these cells could contribute to pathogenesis.

To understand the programming of B cell responses, we studied the response to Salmonella infection, which suppressed GCs while promoting strong EF responses. We discovered a particular cytokine network that suppressed the differentiation of GC B cells and T follicular helper cells, while at the same time enhancing the generation of EF plasmablasts and inflammatory T cells. I will present data on how this network functions as a molecular switch. To understand the role of ABC we studied them in both the immune response to Ehrlichia muris and in the MRL/lpr murine model of lupus, with contrasting results. I will present these data emphasizing that ABC in lupus are a dynamic and heterogeneous population that undergoes clonal expansion and contributes directly to pathogenesis.

Lay abstract I will present data showing how certain types of immune responses that promote lupus are controlled by the immune system. This in turn gives basic insight into lupus pathogenesis and may eventually help in categorizing types of lupus patients and designing and selecting appropriate therapies.

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301 THE LUPUS RISK INDEX (LRI) AS A BIOMARKER IN PATIENTS WITH LUPUS NEPHRITIS
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Background Identification of prognostic and predictive biomarkers in lupus nephritis (LN) is an area of intense study and interest. Non-invasive laboratory markers that associate with the likelihood of responding to therapy and/or assess clinical response in patients with active LN remains an unmet need. The Lupus Risk Index is a score that associates with the risk of developing SLE. It is lowest in Caucasian females and increases in populations with increased risk of developing SLE (African American, Sister’s of patients with SLE), and is highest in SLE. Its components (IgM and IgG anti-DNA antibodies and C1q) are each associated with renal disease (or protection from renal disease).

Objective To assess the LRI as a LN biomarker that predicts a clinical response to induction therapy, or whose early changes may precede a clinical response, or that associates with a clinical therapeutic response.

Methods The LRI was determined on retrospective specimens collected from two LN clinical trials sponsored by the Immune Tolerance Network (ITN): CALIBRATE (n=43) and ACCESS (n=134) as well as healthy controls (n=70). Both trials enrolled subjects with active proliferative LN (Class III,

Lay abstract The Lupus Risk Index is a score that associates with the risk of developing SLE and is lowest in Caucasian females and increases in populations with increased risk of developing SLE (African American, Sister’s of patients with SLE), and is highest in SLE. Its components (IgM and IgG anti-DNA antibodies and C1q) are each associated with renal disease (or protection from renal disease).