203 INSIGHTS INTO LUPUS BIOLOGY FROM INBORN ERRORS OF IMMUNITY: IMMUNOPATHOGENESIS OF STAT1 GAIN-OF- FUNCTION AUTOIMMUNITY

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Background Human genetic variations resulting in immunodeficiency and/or the propensity for autoimmunity represent "experiments of nature" that can advance our understanding of polygenic human diseases. Heterozygous gain- of-function (GOF) mutations in *STAT1* induce immune dysregulation characterized by autosomal dominant chronic mucocutaneous candidiasis (CMC) and the propensity for humoral autoimmunity. However, given widespread expression of STAT1 in immune and non-immune lineages and engagement by multiple cytokine receptors, the immune mechanisms driving breaks in immune tolerance in STAT1 GOF syndrome remain poorly understood. In addition, STAT1 GOF variants are thought to enhance cytokine signaling by increasing total STAT1 protein levels, but the cause of this phenotype has not been identified.

Methods To gain insights into the complex roles for STAT1 in human immunity, we performed mass cytometry using PBMCs from STAT1 GOF patients. Affected subjects were studied prior to treatment with JAK inhibitors, allowing unique insight into the immune landscape of STAT1-driven immune dysregulation. In parallel, we generated a novel murine knock-in strain allowing cell-intrinsic expression of a pathogenic human STAT1 GOF mutation

Results We performed multiparameter immunophenotyping of pediatric STAT1 GOF patients and age-matched controls to identify immune characteristics of STAT1-driven inflammation. Affected patients exhibited expansion of CXCR3-expressing CD4+ T and B cell populations exhibiting surface markers indicative of B cell helper function and extrafollicular activation, respectively. Moreover, relative expansion of these adaptive immune populations correlated with serum autoantibody titers. To study underlying mechanisms, we generated a new *Stat1* GOF transgenic model and confirmed the development of spontaneous humoral autoimmunity recapitulating the human phenotype. Despite clinical resemblance to human regulatory T cell (Treg) deficiency (IPEX syndrome), $Stat1^{GOF}$ mice and humans exhibited normal Treg development and function. Rather, STAT1 GOF autoimmunity was driven by dysregulated STAT1-dependent signals downstream of the type 1 and type 2 interferon (IFN) receptors. Surprisingly, autoimmunity in $Stat1^{GOF}$ mice lacking the type 1 IFN receptor (IFNAR) was only partially ameliorated, whereas loss of type 2 IFN (IFN-γ) signals prevented disease. Strikingly, IFN-γR deletion abolished the known increase in total STAT1 expression resulting in normalization of STAT1-dependent systemic inflammation.

Conclusions Since STAT1 regulates its own transcription, these findings highlight IFN-γ as the critical driver of a feedforward inflammatory cascade in STAT1 GOF syndrome. More broadly, our data provide new insights into the STAT1-dependent cellular mechanisms underlying both rare monogenic and more common polygenic autoimmune diseases, such as systemic lupus erythematosus.

204 IL-4 AS A NEGATIVE REGULATOR OF PATHOGENIC EXTRAFOLLICULAR DN2 B CELLS IN SLE

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Background Pathogenic extrafollicular double negative 2 (DN2) B cells in SLE have a phenotype of $IgD^{-}CD27^{-}Tbet^{+}CD11c^{+}$, and are the precursor of ribonuclear protein (RNP) autoantibody producing B cells. This trajectory of development is promoted by a synergistic effect of type I interferon (IFN) and TLR7-induced activation of B cells at the transitional (Tr) and naïve (NAV) developmental stages. In contrast, IL-4 and IL-4R expression is low in SLE patients and higher expression of IL-4 has been associated with a milder disease course in SLE. The present studies were carried out to determine the mechanism associated with IL-4- mediated B-cell quiescence program *in vitro* in SLE B cells and *in vivo* in the BXD2 mouse model of SLE.

Methods All SLE patients met the American College of Rheumatology 1997 revised criteria and the 2017 ACR/EULAR classification criteria for SLE. Peripheral blood mononuclear cells were analyzed by FACS for surface expression of IL-4R, intracellular IFN-β, and intranuclear T-bet and IRF7. DN2 B cell differentiation *in vitro* was stimulated with or without IL-4 50 ng/ml pre-culture for 1 hr. B-cell phenotypes, autoantibody profiles, and their association with the expression of IL-4R and IFN-β were analyzed in 47 SLE patients. The transcriptomics program associated with B-cell fate decision at the Tr, NAV, and activated naïve (aNAV) stages of B cell development in healthy control (HC) subjects and SLE subjects was analyzed using single cell RNA-sequencing (scRNA-seq) analysis. BXD2 mice were injected weekly with IL-4 complex or carrier anti-IL-4 antibody followed by R848, a TLR7 ligand. Four weeks later, mice were treated an additional IV injection of IL-4 complex or carrier. One week later, mice were sacrificed. The development of autoantibodies, GC B cells, DN2 B cells, and B-cell transcriptome were analyzed.

Results Section scRNA-seq analysis showed that type I interferon (IFN) stimulated genes (ISGs) were upregulated at Tr, rNAV, and aNAV stages of SLE B cells. In contrast, Tr and NAV B cells from SLE patients exhibited downregulation of an IL-4R quiescent gene program consisting of *IL4R*, *BACH2*, and *FCRE2A* (CD23). In HC, aNAV B cells exhibited upregulation of gene signatures of germinal center (GC) and classical memory (cMEM) B cells including *LTB*, *GPR183*, *CD27*, *CD44*, and *CD83*. IKAROS was identified as a top transcription factor associated with the upregulated genes in B cells from HC. In contrast, in SLE, aNAV B cells expressed signature genes of DN2 B cells including *FCRL3*, *FCRL5*, and *ZEB2*. Pseudotime analysis revealed in SLE, 63% of B cells developed into the DN2 pathway compared to only 3% in healthy controls. In contrast, only 14% of SLE B cells developed into the GC and classical memory pathway compared to 20% for healthy controls. IL-4 pre-treatment resulted in a significant increase of the $CD11c^{-}Tbet^{-}$ DN1 subpopulation and a decrease in the $CD11c^{+}Tbet^{+}$ DN2 B cells from SLE