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 extracellular 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-γ stimulation of B cells 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-classified B 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-classified B cells were analyzed by FACS for surface expression of IL-4R-classified B cells were analyzed by FACS for surface expression of IL-4R.

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.

Abstracts

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

1 Lagert Andrea, 2 Kathi Lambert, 3 Chiang Kristy, 4 Shumlak Natali, 5 Quan-Zhen Li, 6 Kelly Huddins, 6 Denny Liggitt, 7 Mohammed Oukka, 8 Troy Torgerson, 9 Jane Buckner, 10 Eric Allenspach, 11 David J Rawlings, 12 Shaun W Jackson. 1 Largent Andrea, 2 Kathi Lambert, 3 Chiang Kristy, 4 Shumlak Natali, 3 Quan-Zhen Li, 6 Kelly Huddins, 6 Denny Liggitt, 7 Mohammed Oukka, 8 Troy Torgerson, 9 Jane Buckner, 10 Eric Allenspach, 11 David J Rawlings, 12 Shaun W Jackson.

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

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Background: Pathogenic extracellular double negative 2 (DN2) B cells in SLE have a phenotype of IgD^CD27^Tbet^CD11c^, and are the precursor of ribonucleic 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 vivo in SLE B cells and in vitro 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-classified B cells 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 IL-4R, 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 LTβ, 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.