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 CD8+ 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), Stat1GOF 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 Stat1GOF 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.
patients. There was a significant correlation of the percent of IL4R-IFNβ+ naïve B cells with SLEDAI, anti-Sm and anti-DNA. In vivo treatment of BXD2 mice with IL-4 significantly blocked R848 induction of CD11c+CD21−B220+ 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 CH223191.

Conclusion Low expression of the IL-4R program and low signal- ing 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 molecular 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 auto- antibodies 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 quiescence 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 exhibited 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 develop- ment of B cells into a pathogenic “DN2” population of B cells. In contrast, in normal controls, this IL-4R programed naïve B cell development promotes a different B-cell develop- mental trajectory that do not result in production of autoanti- bodies. 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 autoanti- bodies that are commonly seen in SLE patients. The molecular mechanism for IL-4R suppression of development of patho- genic B cells in lupus was found to be based on key mole- cules 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.

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 immuno-logic 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 dura- tion) 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 exam- ined 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 Th1 and Th2 expansion; Cluster 3, with reduced levels of innate, naïve B, and Th1 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 dura- tion), 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...