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Dysregulation of innate and adaptive serum mediators precedes systemic lupus erythematosus classification and improves prognostic accuracy of autoantibodies

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ABSTRACT

Systemic lupus erythematosus (SLE) is a complex autoimmune disease with a poorly understood preclinical stage of immune dysregulation and symptom accrual. Accumulation of antinuclear autoantibody (ANA) specificities is a hallmark of impending clinical disease. Yet, many ANA-positive individuals remain healthy, suggesting that additional immune dysregulation underlies SLE pathogenesis. Indeed, we have recently demonstrated that interferon (IFN) pathways are dysregulated in preclinical SLE. To determine if other forms of immune dysregulation contribute to preclinical SLE pathogenesis, we measured SLE-associated autoantibodies and soluble mediators in samples from 84 individuals collected prior to SLE classification (average timespan = 5.98 years), compared to unaffected, healthy control samples matched by race, gender, age (±5 years), and time of sample procurement. We found that multiple soluble mediators, including interleukin (IL)-5, IL-6, and IFN-γ, were significantly elevated in cases compared to controls more than 3.5 years pre-classification, prior to or concurrent with autoantibody positivity. Additional mediators, including innate cytokines, IFN-associated chemokines, and soluble tumor necrosis factor (TNF) superfamily mediators increased longitudinally in cases approaching SLE classification, but not in controls. In particular, levels of B lymphocyte stimulator (BLyS) and a proliferation-inducing ligand (APRIL) were comparable in cases and controls until less than 10 months pre-classification. Over the entire pre-classification period, random forest models incorporating ANA and anti-Ro/SSA positivity with levels of IL-5, IL-6, and the IFN-γ-induced chemokine, MIG, distinguished future SLE patients with 92% (±1.8%) accuracy, compared to 78% accuracy utilizing ANA positivity alone. These data suggest that immune dysregulation involving multiple pathways contributes to SLE pathogenesis. Importantly, distinct immunological profiles are predictive for individuals who will develop clinical SLE and may be useful for delineating early pathogenesis, discovering therapeutic targets, and designing prevention trials.

1. Introduction

Systemic lupus erythematosus (SLE) is a clinically and serologically heterogeneous systemic autoimmune disease which causes significant morbidity and early mortality, especially in young women and minorities (1). Immune dysregulation in the form of pathogenic autoantibodies and chronic inflammation contributes

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to a wide range of clinical manifestations, including skin rashes, arthritis, and life-threatening renal and/or central nervous system damage [1]. A number of antinuclear autoantibody (ANA) specificities have been shown to accumulate prior to SLE classification [2–4]; preclinical use of hydroxychloroquine may abrogate autoantibody accumulation and delay clinical disease onset [4]. Early intervention is an attractive approach to SLE treatment. However, our understanding of pathogenic mechanisms in preclinical SLE is inadequate. Closing this knowledge gap would improve our ability to identify individuals with preclinical SLE, define windows of opportunity for early intervention, and facilitate the development of pathway-targeted treatments.

Current biomarkers in preclinical SLE have limited utility for forecasting the transition to classified disease [2,3,5]. Although SLE-associated autoantibody specificities such as anti-dsDNA, anti-spliceosome and anti-Ro/SSA, accumulate in SLE patients years before classification [3], their presence is not sufficient to predict SLE. ANAs are also found in sera from patients with other systemic rheumatic diseases [6], and from healthy individuals who do not go on to develop SLE, including some unaffected family members of SLE patients [7], and up to 14% of the general population [8]. Because individuals may remain healthy despite being ANA-positive, ANA positivity alone is likely not the sole pathogenic driver of SLE [2,9,10]. In addition to ANA positivity, the dysregulation of various immune pathways driven by soluble mediators may contribute to the development of clinical disease. High expression of type I interferon (IFN)-related genes has been associated with SLE, yet an elevated IFN signature is not present in all patients [5]. Evidence stemming from lupus-like animal models and SLE patients suggests that breaks in tolerance leading to the activation and persistence of autoreactive B cells arise from amplified crosstalk between innate and adaptive immunity [11,12]. Key mediators of such crosstalk, including Th-type cytokines IFN-γ (Th_1), interleukin (IL)-4 and IL-5 (Th_2), and IL-17 and IL-21 (Th_{17}) facilitate lymphocyte recruitment to germinal centers [13–15] and pathogenic autoantibody production [16,17] with the help of T-follicular helper (T_{fh}) cells [17]. We have recently demonstrated that type II IFN (IFN-γ) becomes elevated prior to and concurrent with the development of lupus-associated autoantibodies [18]. The tumor necrosis factor (TNF) superfamily member B lymphocyte stimulator (BLyS), secreted in response to type I and type II IFNs [19,20], further supports and propagates autoantibody production as a survival factor for self-reactive B-lymphocytes [21]. In addition to driving the production of pathogenic autoantibodies, these mediators also contribute to inflammation associated with SLE disease flare [22] and organ damage [23]. Although these mediators contribute to SLE disease activity, their role in preclinical autoimmunity and transition to clinical disease are not well understood.

No single factor or mechanism is likely sufficient to explain the complexity and heterogeneity of SLE pathogenesis; thus a multivariate, longitudinal approach is warranted to delineate mechanisms of early disease pathogenesis and discern unique parameters that forecast SLE classification. In the current study, we leveraged longitudinal serum samples from the Department of Defense Serum Repository (DoDSR) to compare levels and determine temporal relationships between autoantibodies and immune mediators from multiple immune pathways in individuals who subsequently developed SLE compared to matched, healthy controls. Our findings shed light on potential mechanisms of early preclinical SLE immunopathogenesis, whereby dysregulation of immune mediators occurs prior to and concurrent with autoantibody accumulation, and is amplified leading up to SLE classification. Further, this study informs the design of reliable and sensitive tools to predict SLE onset. Such tools can be used to identify high risk patients in need of rheumatology referral and enrollment in prospective, preclinical intervention studies, as well as inform the development of novel treatment strategies to avert or delay tissue damage that often accompanies transition to classified disease [24–27].

2. Materials and methods

2.1. Study population and serum samples

Experiments were performed in accordance with the Helsinki Declaration and approved by the Institutional Review Boards of the Oklahoma Medical Research Foundation and the Walter Reed National Military Medical Center. Samples were obtained from the DoDSR. Demographic and clinical information, including medication history and American College of Rheumatology (ACR) criteria for SLE classification, were extracted from medical records by study personnel. All patients with available serum samples covering periods before and at/after SLE classification (n = 84) were selected from a cohort comprised of 130 previously identified individuals [2,28] and 75 newly identified individuals with classified SLE (≥ 4 ACR criteria for SLE [29,30]). Cases were compared to healthy controls matched by race, sex, age (±5 years), and time of sample procurement relative to SLE disease classification, as well as sample availability (n = 86; Supplemental Table 1). Individuals selected as matched healthy controls had no signs or symptoms of autoimmune disease in their medical record during the time span assessed. In total, 416 samples were analyzed (246 from cases and 170 from controls). Cases had an average of 2.96 available samples (range, 2–3), and controls had an average of 2 available samples (range, 1–3). For sequential longitudinal analysis, samples from SLE cases and their matched controls were divided into four time periods relative to SLE classification, such that each time period included approximately 60 case samples (range, 61–63) (Supplemental Fig. 1).

2.2. Soluble mediator and autoantibody assays

Serum levels of BLyS (R&D Systems, Minneapolis, MN) and a proliferation-inducing ligand (APRIL) (eBioscience/Affymetrix, San Diego, CA) were assessed using enzyme-linked immunosorbent assay (ELISA) per manufacturer's protocol. Normalized fluorescence intensity values for an additional 30 immune mediators, including cytokines, chemokines, and soluble TNFR superfamily members (Supplemental Table 2), were determined by xMAP multiplex assays (eBioscience/Affymetrix) [18]. After performing quality control as described previously [31], four mediators (IFN-α, TNF-α, IL-10, and IL-15) were excluded from further analysis due ≥ 50% of cytokine measurements falling below the lowest level of detection [32]. The average inter-assay coefficient of variance (CV) of the assays performed in this experiment was 10.5%, comparable to the previously reported CV values (10%–14%) for multiplexed bead-based cytokine assays [33,34]. Intra-assay precision was high, with an average CV of <10% for duplicate wells in each 30-plex assay. The BioPlex 2200® system (Bio-Rad Technologies) was used to simultaneously detect levels of multiple autoantibody specificities within a single serum sample: dsDNA, chromatin, Ro/SSA, La/SSB, Sm, SmRNP, and RNP [7,35]. Semi-quantitative values for anti-dsDNA were reported as IU/mL (positive ≥ 10 IU/mL). All other autoantibody specificities were reported in autoantibody index (AI) units based on the fluorescence intensity (range 0–8) using the manufacturer-specified positive cutoff (positive ≥ 1 AI). Factor XIIIb levels were evaluated as a quality control measure, serving as both a serum confirmation and an indicator of sample integrity.