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Editorial

Immune phenotypes in individuals positive for antinuclear antibodies: The impact of race and ethnicity

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Using an impressive array of immunophenotyping assays, Slight-Webb et al[1] provide important new information on key issues in the pathogenesis of systemic lupus erythematosus (SLE): the role of antinuclear antibodies (ANAs); the impact of race and ethnicity on disease susceptibility; and the properties of immune cells regulating autoimmunity. Although the study involves only a limited number of patients, the extensive immunophenotyping provides intriguing evidence for a unique immune profile that may determine the transition from normal to aberrant immunity.

As is well known, ANA production is a prominent feature of SLE and related autoantibody-associated rheumatic diseases (AARDs) such as Sjogren syndrome, myositis, and systemic sclerosis. These antibodies bind to DNA, RNA as well as protein complexes of DNA and RNA.[2] Importantly, immune complexes (ICs) between ANAs and their cognate antigens can stimulate the production of type 1 IFN and other cytokines; this stimulation occurs following the uptake of ICs into innate immune cells and the interaction of the cargo DNA or RNA with internal nucleic acid sensors. These receptors, which include Toll-like receptors, are part of an internal host defense recognizing nucleic acids aberrantly present in the cytoplasm from infection or cell stress.

Although some ANAs can have immune activity, the expression of ANAs appears to be widespread in humans. Indeed, as many as 20% of the otherwise healthy individuals can express an ANA as detected by the usual serological assays.[3] Among these assays is the immunofluorescence assay (IFA) using HEp-2 cells, long considered the criterion standard for ANA detection. ANAs can also be detected by ELISAs as well as addressable laser bead immunoassays, which are increasingly popular for these determinations because of their high throughput.[2]

The high frequency of ANA positivity in the general population, especially women, is poorly understood although it appears to be rising, perhaps related to environmental factors.[3] Importantly, although the target antigens recognized by ANAs in patients with SLE and other AARDs are well defined biochemically, the antigens recognized by the otherwise healthy population are, in general, unknown. As a screening test for early diagnosis or prevention, the ANA assay has great limitations because the false-positivity rate is so high. Despite the high frequency of ANAs in the population, SLE affects only about 0.1% of people.

Although most ANA-positive individuals will never develop any disease, ANA production is an early event in SLE. ANA production can precede signs and symptoms of disease by 5 or more years, with more detailed serological analysis demonstrating increasing production of antibodies to nuclear antigens such as DNA, Sm, RNP, Ro, and La.[4] This stage of disease can be called preautoimmunity because symptomatology is not manifest. Along with more diverse ANA production, disturbances of cytokine production can also develop during this stage, perhaps related to the role of ANA ICs in driving cytokine production (Fig 1).

Analysis of immune features of ANA-positive individuals without clinical disease is, thus, an important approach to chart the progression of immune system changes leading to autoimmunity.[5-7] Because ANAs are the defining feature of SLE and other AARDs, these biomarkers provide a useful probe for mechanisms despite their flaws as screening biomarkers. For their study, Slight-Webb et al performed immunophenotyping of 3 populations of women: ANA-negative healthy women, ANA-positive healthy women, and women with SLE. In view of the increased frequency of SLE and more severe disease in African Americans (AAs) compared with European Americans (EAs), AA and EA populations were separately analyzed to explore potential reasons for the major differences in disease frequency and course in racial and ethnic populations; these differences include more robust responses to RNA-protein complexes. Each group had 12 patients.[1]

The immunophenotyping used state-of-the-art techniques: single-cell mass spectrometry, flow cytometry, next-generation RNA sequencing, multiplex cytokine profiling, and phospho-signaling analysis. In addition, serological testing assessed responses to common viruses such as cytomegalovirus, EBV, and herpes simplex virus. This is likely the most detailed analysis ever performed to chart the pathways to autoimmunity.

Because the phenotyping was very extensive, the study produced many interesting findings; 2 are especially notable. The first finding is that, compared with EA healthy individuals and EA patients with SLE, EA ANA-positive healthy individuals had a unique phenotype that was also not observed in AA cohorts. On the basis of T, natural killer, and natural killer T-cell numbers, cytokine levels, and patterns of T-cell signaling, this phenotype is consistent with suppression, suggesting an effect on the progression to clinical autoimmunity of EA individuals in a way distinct from AA individuals. The cell populations targeted

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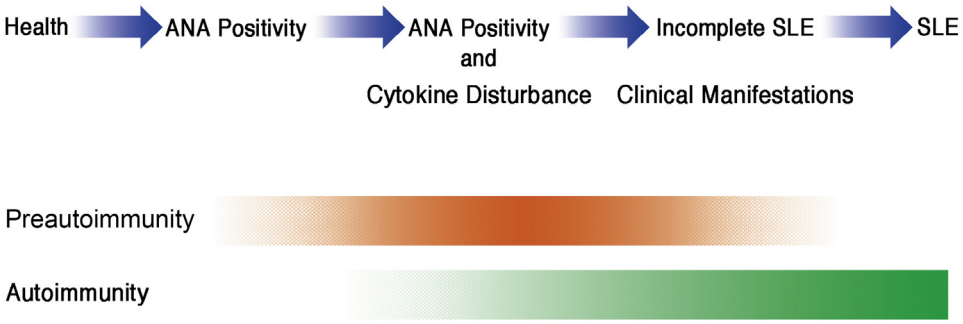

FIG 1. The evolution of SLE. As suggested by current studies, the development of SLE can occur in a stepwise fashion that begins with ANA positivity. Although ANA positivity is common in the general population, in some individuals, increased cytokine production develops, possibly because of the role of ICs of ANAs with DNA and RNA in stimulating cytokine production. Subsequently, clinical manifestations develop although the findings are not sufficient for classification as SLE; such individuals have an incomplete form of SLE, which can be defined as fewer than 4 American College of Rheumatology classification criteria. Eventually, in some individuals, accrual of clinical and laboratory findings allows classification (or diagnosis) with SLE as demonstrated by 4 or more of the classification criteria. The boundary of preautoimmunity is not clear although the confluence of ANA production and cytokine disturbance appears reasonable. In this regard, it is possible that properties of ANAs change over time (eg, increased affinity), transitioning from nonpathogenic to pathogenic. Steps along the progression likely relate to genetics as well as environmental exposures, with infection a possible trigger for both ANA production and increased cytokine responses. The boundaries between health, preautoimmunity, and autoimmunity is not clear although intense and frequent laboratory monitoring of populations would lead to more precision.

by the putative suppression are not clear, however, and, although some of the changes resembled those of immune suppression from virus infections, levels of antibodies to viruses were nevertheless intact despite this phenotype.

A second notable observation relates to the analysis of cytokines. These studies showed that stem cell factor was the only cytokine whose levels were increased in both AA and EA patients with SLE compared with ANA-negative and ANA-positive healthy controls. This finding is of interest because stem cell factor has not been a major focus of attention in studies on SLE pathogenesis. However, consistent with many other studies on cytokine disturbances in SLE, Slight-Webb et al found strong IFN signatures in various immune cell populations of patients, with elevation in IFN-associated mediators more evident in AA patients.

In studies of this kind, the assay used for ANA detection can influence the populations analyzed. This study used the BioPlex 2200 assay for initial screening. This addressable laser bead immunoassays measures antibodies to 11 different autoantigens and includes specificities relevant to SLE as well as myositis, Sjogren syndrome, and systemic sclerosis. As such, this approach differs from other studies in which an IFA represents the initial screen. Most healthy subjects who are IFA positive lack antibodies to known antigens relevant to AARDs. In general, the frequency of false-positive results with the BioPlex 2200 is lower than that of the IFA,[8] suggesting possible differences in the healthy subjects in this study compared with studies in which the IFA was used for ANA screening.

Another potential difference relates to the source of patients. Slight-Webb et al recruited patients from health fairs, whereas other studies have involved subjects evaluated for a positive ANA but found not to have a connective tissue disease.[7] A questionnaire was used to rule out a connective tissue disease in the Slight-Webb et al study. Given the complexity of the lupus phenotype, matching populations in terms of demographics and serology is challenging but Slight-Webb et al tried to get as close as possible to comparable populations. Even with the best matching, however, the study provides only one point in time in a process that likely unfolds over many years.

The development of biomarkers to assess preautoimmunity is key for strategies for early detection and disease prevention, but these biomarkers must be actionable. The ANA itself is inadequate for this purpose because the number of false-positive responses (especially in young women) is far too great to allow meaningful detection. A multiplex ANA is perhaps better but, even with assays to defined nuclear antigens, the frequency of false-positives is high. Although the study of Slight-Webb et al suggests the value of in-depth immunophenotyping, the analysis involved 55 cell populations and 51 cytokines. Reducing the number of analytes to the most sensitive and specific markers will be an important challenge for the future.

By expanding the analysis of at-risk populations to characterize AA and EA populations, the study by Slight-Webb et al is an important step toward personalized medicine. As shown in many studies, AA patients with SLE are especially prone to renal disease and poor outcomes. Although many factors are likely contributory, the worse outcome of AA patients for many diseases including COVID-19 infection has suggested more systemic factors, perhaps related to racism, which remains persistent in the United States.[9,10]

As studies on the transition from preautoimmunity to autoimmunity advance, it will be important to incorporate other variables such as socioeconomic status, nutrition, and the complex ensemble of factors related to race and ethnicity. Only with a more complete accounting of the dimensions of personhood will it be possible to realize the goal of personalized medicine and reduce the health care disparities that make SLE such a serious medical problem.

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