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Subduing Lupus: Can Preclinical Autoimmune Disease Be Arrested?

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Systemic lupus erythematosus is a complex and heterogeneous systemic autoimmune disease in which progressive immune complex-mediated organ damage occurs that is debilitating and sometimes fatal. It arises from a combination of genetic factors and environmental exposures, many of which are not well understood at this time. Like other autoimmune diseases, the onset of clinical symptoms of lupus is preceded by years of asymptomatic autoimmunity manifested by the presence of specific and nonspecific autoantibodies and evidence of immune dysregulation. Since irreversible organ damage has often occurred by the time of initial diagnosis, it is critically important that we better understand the “preclinical” stage of lupus so that treatment can be started earlier. Besides preventing organ damage and death, intervention at these early time points holds the promise of reversing autoimmune phenotypes and restoring tolerance, thereby delaying disease onset, perhaps indefinitely.

Antinuclear antibodies (ANA) are associated most commonly with lupus, being present in nearly all cases of diagnosed disease. But a positive ANA is neither specific nor prognostic, as most people with these antibodies will never develop lupus or any other autoimmune or inflammatory disease. Therefore, it is of great interest and importance to understand what factors give a positive ANA predictive value for lupus. Some knowledge has been gleaned from studying later stages in disease progression, including the state termed undifferentiated connective tissue disease (UCTD) in which some clinical symptoms of connective tissue disease are present, though they are not specific for a distinct clinical entity, or the state termed preclinical, incomplete, or potential lupus in which some specific features of lupus are present but insufficient in number to classify the patient with this disease. From these studies, it can be inferred that female sex, homogenous ANA pattern, certain ANA specificities (anti-dsDNA or anti-Smith), anti-cardiolipin antibodies, multiple autoantibody reactivities, and multiple clinical features of lupus are predictive of transition to clinical lupus [reviewed in (1)]. Another biomarker associated with lupus transition is increased ratios of Th17 cells to regulatory T cells (2), which suggests that skewing in inflammatory and regulatory immune mechanisms promotes disease development. Whether other biomarkers in individuals with incomplete lupus, including interferon gene expression signatures (3) and autoreactivity to synthetic autoantigen surrogates (“peptoids”) that are

more specific for lupus than the ANA (4), predict disease progression await longitudinal prospective analyses.

In this issue of *Arthritis & Rheumatology*, Slight-Webb *et al* seek to further distinguish the factors that determine lupus progression or arrest. They identify at-risk individuals at state health fairs based on their serum being positive for one of the ANA specificities associated with classifiable autoimmune disease (dsDNA, chromatin, SSA/Ro, SSB/La, Sm, Sm/RNP, RNP, ribosomal P, Scl-70, centromere B, and Jo-1). They then perform cross-sectional analyses comparing the immune phenotypes of these individuals with those of ANA-negative healthy controls or lupus patients matched for age, sex, ethnicity, and body mass index. Although this strategy has the advantage of selecting for individuals more likely to develop an autoimmune rheumatic disease, it does not clearly identify those at risk for progression to lupus. When comparing demographics alone, the only factor they found to be linked to ANA-positivity was female sex, similar to previous studies (5).

Since cytokines are important in regulation of immune responses and cytokine profiles are known to differ during lupus flares compared to periods of inactive disease, they next examined differences in patterns of cytokine production across the three groups. They identified three distinct cytokine clusters, one downregulated in both ANA+ and lupus groups, one upregulated in the lupus group and sometimes also in the ANA+ group, and one upregulated only in the ANA+ group, as well as one cytokine that was decreased only in patients with lupus. What does the identity of these cytokines and their patterns tell us about lupus progression?

Some of the mediators that were downregulated are involved in regulation of metabolism (resistin and leptin). Recent studies have shown that medications approved for treatment of insulin-resistant diabetes may be beneficial in lupus (6, 7), suggesting that dysregulation of metabolic pathways is involved in lupus pathogenesis. Others were involved in inflammation and endothelial cell activation. Why these mediators are downregulated is not clear. Is it because of transcriptional effects induced by an evolving autoimmune response? Are they being internalized by target cells? Are the cells that are producing them being sequestered in tissues? Could reduction in cell numbers account for these differences? Are these factors specifically involved in the development of lupus or do they represent the pathological changes associated with development of any inflammatory autoimmune disease?

Approximately half of the cytokines were upregulated in both patients with lupus and subjects with positive ANAs, with the subjects with positive ANAs having lower mean levels compared to subjects with lupus. This finding suggests that these cytokines are driven by the formation of pathogenic immune complexes that increase in number as disease onset approaches. Alternatively these cytokines may be driving the production of autoantibodies, and their higher levels as subjects move from autoimmunity to disease onset may represent progressive cytokine-driven immune dysregulation. The finding that a subset of cytokines, including the IFN α , IFN β , BLYS, IL-12p40, and SCF/c-kit ligand, were upregulated only in subjects with lupus was surprising and suggested that either these cytokines are released closer to the time of transition to disease onset or that their production results from established disease. The authors postulate that the reason that inflammatory cytokine profiles

are stronger in patients with lupus than in healthy ANA-positive subjects is because lupus patients have reduced levels of the regulatory cytokine IL1RA, and consequently reduced IL-1RA/IL1 β ratios compared to asymptomatic subjects with positive ANAs, and thus may be less able to regulate the inflammatory responses induced by pathogenic immune complexes. It is interesting that one of the primary sites of IL1RA synthesis is adipose tissue (8) and its production is induced by leptin (9), again linking cytokine dysregulation in lupus to altered metabolic processes.

Finally, some cytokines were upregulated only in ANA+ subjects (MIP-1b, CD40L, and IL-8). Multiple scenarios can be envisioned to explain this. Do these cytokines suppress the evolving immune dysregulation associated with disease progression? Are they associated with progression to alternative ANA-associated autoimmune diseases? Are they suppressed by the treatments used in lupus? The answers to these questions cannot be resolved until larger longitudinal studies are performed. Despite the multiple possible ways that these various groups of cytokines might participate in disease evolution and pathogenesis, the authors were able to identify a four-cytokine panel including BLYS, IL-5, G-CSF, and IL-2 that could distinguish ANA+ healthy individuals from ANA- healthy individuals or lupus patients, thereby suggesting a method by which ANA+ individuals at higher risk of evolving to clinical disease could be identified, based on their deviation from this profile.

The authors also identified dysregulation in the cellular components of the peripheral blood in healthy individuals with antinuclear antibodies. Total numbers of PBMCs were reduced, suggesting that the expansions of monocytes, memory B cells, and plasmablasts that the authors identified were due to contractions of other cell populations within the peripheral blood compartment rather than absolute increases in their numbers. Specifically, the absolute numbers of four cell populations were found to be decreased: CD85j+T cells, CD4+ T cells, NK cells, and transitional B cells. Interestingly all of these cell subsets are known to harbor regulatory cells, but whether the regulatory cells in these populations were specifically reduced was not determined. This hypothesis is intriguing however, as their depletion could enable autoreactive B cells producing antinuclear antibodies to expand, suggesting both a mechanism for disease evolution as well as a potential intervention to arrest this process. Alternatively, these population changes may be due to contraction of effector CD4+ and NK cells due to enhanced regulatory mechanisms associated with the preclinical state, which may in turn prevent progression to disease in these individuals.

Finally, the authors found increased basal STAT5 phosphorylation in monocytes and B cells of subjects with positive ANAs, with modest though significant increased phosphorylation of STAT1 and STAT3 in both monocytes and B cells and of STAT5 in monocytes in response to IFN α stimulation. These data suggest that these cells are primed *in vivo* by the altered cytokine milieu associated with ANA-positivity and that they are hyperresponsive to additional inflammatory signals to which they might be exposed. Therefore, even though effector B cells and monocytes do not appear to be expanded in ANA+ subjects, they are poised to respond to inflammatory cytokines such as IFN α , and perhaps when this cytokine is produced closer to the time of diagnosis, they generate the pathogenic dysregulated autoimmune responses that drive the development of disease manifestations.

In conclusion, the investigators cite the absence of elevated levels of type I IFNs, BLYS, IL-12p40, and SCF/c-kit ligand and the presence of increased IL-1RA:IL-1 β ratios as protective factors that prevent the progression of benign autoimmunity to clinical disease. Conversely, increased expression of these cytokines or reduced IL-1RA:IL-1 β ratios might portend impending disease development. Only with carefully conducted longitudinal analyses of these subjects can the relevance of these biomarker panels be ascertained with confidence and authentic pathogenic mechanisms that lead to lupus onset distinguished from artifacts or alternative mechanisms associated with development of other ANA-positive autoimmune diseases.

One can envision a day when, by utilizing genetic data, autoantibody profiling, cytokine arrays, immunophenotyping, and perhaps additional measures of immune dysregulation that have yet to be determined, individuals who are at risk to develop lupus can be identified and stratified based on their likelihood of developing pathological autoimmunity within a certain time frame (Figure 1). Clinical trials have already begun in preclinical type I diabetes and rheumatoid arthritis to stop disease before it starts (11, 12). Perhaps those conducted in lupus will not be far off. This is certainly the goal of studies such as those by Slight-Webb *et al*, and their findings reported in this month's issue of *Arthritis & Rheumatology* move us one step closer.

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Figure 1. Stages of development of systemic lupus erythematosus and factors predictive of progression of disease

Genetic factors contribute to disease risk, with gene variants with moderate or strong effects shown (10). Numerous other gene variants with weaker effects as well as rare variants likely also contribute to risk. After autoantibodies develop, immune dysregulation can be identified in the form of cytokine dysregulation, immune cell dysregulation, and altered cell signaling. Whether these alterations influence the transition to incomplete lupus/UCTD and classifiable lupus is not yet known, and awaits longitudinal experiments of the natural history of disease for resolution.