PUBLIC HEALTH IN RHEUMATOLOGY: ARE SCREENING AND PREVENTION POSSIBLE?

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Purpose

The purpose of this program will be to discuss the concept that autoimmune rheumatic diseases occur in stages that may be amenable to screening and preventative therapies.

Overview

The following topics will be presented:

1. Autoantibodies appear several years before symptoms in both Systemic Lupus Erythematosus (SLE) and Rheumatoid Arthritis (RA).
2. The prevalence and diversity of autoantibodies increases in the two years prior to symptoms and precedes rising concentrations of inflammatory markers.
3. Screening the general population for rheumatic diseases is difficult and will require a combination of genetic, environmental, and serological factors.
4. Autoantibodies alone are insufficient to screen for disease in the general population.
5. Clinical trials to prevent RA and SLE are being planned.

Objectives

1. Understand the concept that autoantibodies are present many years before the onset of symptoms of RA and SLE.
2. Know that the pre-clinical period of autoimmune disease is characterized by changes in inflammatory cytokines in addition to a broad array of autoantibodies.
3. Be able to describe the utility of commonly used serological tests as screening tests for RA and SLE.
4. Understand that effective screening for RA and SLE in the general population will require a multifactorial approach.
5. Describe the rationale for prevention trials in RA and SLE.

Introduction

Autoimmune diseases often represent a challenge to physicians. As individual diagnoses, these conditions are relatively rare and practitioner unfamiliarity can lead to delays in diagnosis and treatment. As a group, however, autoimmune disorders are **not** rare. It has been estimated that approximately 5 to 8% of the US population suffers from an autoimmune condition at any one time, including rheumatoid arthritis, systemic lupus erythematosus, type I diabetes, multiple sclerosis, psoriasis, and inflammatory bowel disease[1]. However this number does not tell the whole story. Patients with these conditions have reduced lifespans. Therefore, the lifetime risk of autoimmune disease is much higher. A study from the Mayo Clinic looked at inflammatory rheumatic diseases such as RA, SLE, and vasculitis[2]. They estimated that one and 12 women and one in 20 men will develop an inflammatory rheumatic disease in their lifetime. While these conditions can strike at any time, they are most common in young adulthood and can lead to a life long disability. They carry high societal and financial cost. It is therefore a significant public health question to develop better diagnostic tools that will identify patients with autoimmune diseases before they become disabled, and at a time when prevention may be possible.

The concept of disease prevention is common in medicine. For some conditions such as childhood communicable diseases, the entire population is considered to be at risk. The risks of vaccination are low and the benefits are very high therefore screening of individuals is not done and universal preventative measures are suggested. In other situations, we do screen for people at risk. Hyperlipidemia is a risk factor for coronary artery disease. Hypertension is a risk factor for stroke and renal disease. HPV infection is a risk factor for cervical cancer. In each of these cases, the US Preventative Services Task Force has advised population-based screening tests or preventative strategies[3]. In the case of autoimmune diseases, the situation is less clear. The World Health Organization recommendations on disease screening - now nearly 40 years old - are still relevant[4]. In part, they state that a latent or preclinical stage of the disease should be detectable, a test or examination for the condition that defines the preclinical state should exist, and that the natural history of the disease should be adequately understood. Both laboratory and clinical investigations have been done to meet these requirements for most, if not all, autoimmune diseases. This discussion will be confined to rheumatoid arthritis RA) and systemic lupus erythematosus (SLE), the two inflammatory rheumatic diseases for which the most information is available.

Natural History of Autoimmune Rheumatic Diseases

In order to understand the preclinical state of RA or SLE, it is important to know how these diseases are diagnosed. Unfortunately, there are no ”gold standard” tests for diagnosis. Instead, these diseases are classified according to criteria established by expert opinion. The current criteria for RA and SLE are at the end of the protocol[5,6]. In general, they require the presence of characteristic autoantibodies as well as evidence of specific immunopathology. In the case of RA, that is symmetric, inflammatory synovitis, with a

predilection for small joints. For SLE, the immunopathology takes many forms. It can involve the skin, joints, or serosal surfaces, and renal, neurological and hematological systems. Adding to the diagnostic confusion is the fact that over the years these criteria have changed. The most recent versions emphasize early case finding and are therefore more sensitive and less specific than earlier ones[7-10].

In Europe, the search for preclinical versions of these conditions has been facilitated by the existence of national registries of patients as well as biobanks of blood specimens taken from blood donors throughout their lifetimes, and from mothers at the time of delivery. In the United States no such national registries or biobanks exist, with one exception. Members of the US Army and Navy have serum stored upon entry into military service and at approximately two-year intervals. Several groups have used this Department of Defense (DoD) serum repository and associated clinical data from the Walter Reed Army Medical Center Rheumatology Clinic to study pre-clinical autoimmunity.

Autoantibodies are Present Before Symptoms of Disease

In 2003, Arbuckle, *et al*. at the Oklahoma Medical Research Foundation (OMRF) published data on 130 servicemen and women who had developed SLE while on active duty[11]. The group consisted of 47 men and 83 women, and was 62% African-American, reflecting the demographics of the US military. A total of 633 serum samples were available from these subjects, distributed before and after diagnosis. All subjects had at least one pre-diagnosis sample. These serum samples were tested for anti-nuclear antibodies (ANA) as well as a panel of specific autoantibodies associated with SLE. 88% of subjects had at least one autoantibody present before diagnosis. On average, the first autoantibody was present 3.2 years before diagnosis and 2 years before the onset of symptoms. The earliest appearance of serological autoimmunity was 9.4 years before diagnosis. The specific antibodies appeared to cluster in time. ANA, anti-Ro, anti-La, and anti-phospholipid appear first (Figure 1). The autoantibodies that are more specific the SLE, such as anti-double stranded DNA, and anti-Sm appeared much closer to the time of diagnosis, suggesting that an evolution, or spreading, of the autoimmune response that pushed these patients into clinical autoimmunity.

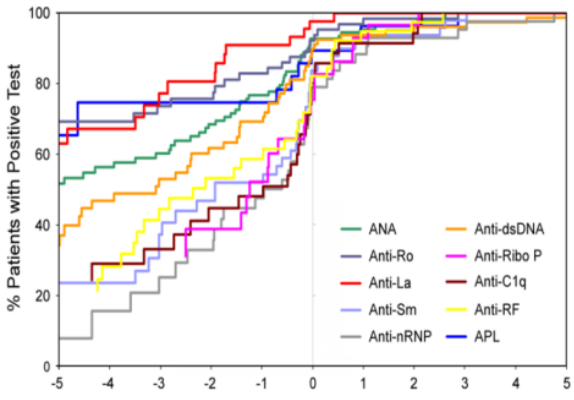


Figure 1. Prevalence of autoantibodies prior to the diagnosis of lupus. Reproduced from Arbuckle, *et al.*

These findings were replicated by Swedish investigators using banked blood specimens from 22 lupus patients[12]. Again, anti-Ro was the earliest autoantibody detected, seen an average of 6.6 years before the onset of symptoms. ANA was the most common positive serological test, seen in 73% of all subjects.

The clinical data associated with the military subjects in the DoD repository offer clues to the natural history of lupus[13]. The OMRF investigators were able to review the charts of

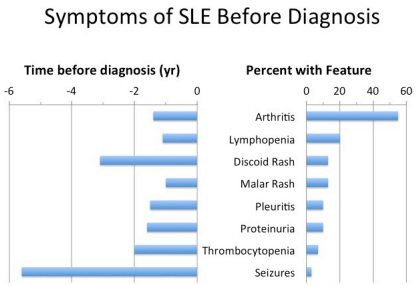


Figure 2. Prevalence of the first signs and symptoms of SLE and the median time of appearance.

these subjects and look for the onset of the different clinical features that ultimately led to lupus classification. Only 20% of cases presented with four or more features on first presentation allowing them to be immediately classified with lupus. The rest of the subjects had a gradual onset of signs and symptoms. Over half of the subjects had only one criterion at the first presentation to a health care provider. Arthritis was the most common presenting feature, seen in 53% of cases. Discoid rash was often seen more than 3 years before final disease classification, although it was seen in only 10-20% of cases. When the investigators compared the timing of certain autoantibodies to clinical features associated with those serologies, it was clear that the antibodies preceded the clinical findings. IgG-Rheumatoid Factor (RF) preceded inflammatory synovitis in 15 of 16 cases; anti-DNA antibodies preceded lupus nephritis in 35 of 38 cases. Together, these data paint a picture of serological autoimmunity in lupus that begins years before clinical symptoms. The serological response becomes more significant with more antibodies, including disease-defining ones at a time when clinical features start to accumulate. This suggests a window when accurate screening tests and possible prevention could be applied.

Antibodies to Citrullinated Proteins are Present Years Before RA Symptoms

The understanding of the natural history of rheumatoid arthritis has been greatly aided by the characterization of a group of autoantibodies termed anti-citrullinated peptide/protein antibodies, or ACPA[14]. Post-translational enzymatic conversion of arginine to citrulline in certain proteins makes them immunogenic. The typical targets ACPA are citrullinated peptides from fibrinogen, vimentin, α-enolase, collagen, and fillagrin. In clinical practice, a synthetic proprietary cyclic peptide antigen is used (anti-CCP) as the target for many of these antibodies. While both ACPA and the traditional rheumatoid factor have similar sensitivities for rheumatoid arthritis, the ACPA or anti-CCP is much more specific making it a better candidate for a screening test[15].

Using banked serum from individuals who later developed rheumatoid arthritis, both Dutch and Swedish investigators looked for ACPA prior to the development of RA symptoms. 83 RA patients who had been part of the Northern Sweden Health and Disease Study or maternity cohorts from northern Sweden were analyzed for the presence of rheumatoid factor or anti-CCP prior to the onset of RA symptoms[16]. IgM-RF was found in 19.3% and anti-CCP in 33.7% of patients who had pre-disease serum samples obtained a median of 2.5 years before the onset of their symptoms. The earliest anti-CCP positive sample was obtained nine years before symptoms. Both the percentage of patients with positive serum samples, and the titer of anti-CCP rose in the year and a half prior to diagnosis. Similarly, 39 of 79 Dutch patients with RA had either an IgM-RF and/or anti-CCP in serum samples obtained a median of 4.5 years before symptoms[17]. The specificity of anti-CCP was confirmed in that it was only found in 0.6% of 2,138 control serum samples.

Investigators at the University of Colorado have used the DoD serum repository to study rheumatoid arthritis. They examined 83 cases of RA where pre-diagnosis serum samples were available[18]. 57% were positive for rheumatoid factor and 61% were positive for anti-CCP before diagnosis. Although gender, race, and age were not associated with autoantibody positivity, there was an interesting relationship between age of the patients and the duration of seropositivity before diagnosis. For subjects aged 20-29, the median duration of anti-CCP positivity prior to diagnosis was 2.8 years whereas in subjects aged 40-49 it was 9.2 years. Anti-CCP positivity before diagnosis was strongly associated with the development of erosive findings on radiographs with an odds ratio of 4.64 (1.17-12.63; $p< 0.01$). This association of anti-CCP with erosive disease has been corroborated by animal models of collagen-induced arthritis showing that antibodies to citrullinated proteins enhance joint inflammation[19], as well as additional human studies documenting the relationship between anti-CCP positivity and erosions on radiographs[20]. Thus, anti-CCP should be thought of not only as a predictive of disease, but a prognostic factor for disease severity.

The Presence of Autoantibodies Predicts Pathological Immune Responses in the Pre-Clinical Phase of Autoimmune Disease

Several studies have looked at other serum biomarkers to help characterize the pre-clinical phase of auto immunity. Again using the DoD serum repository, Sokolove, *et al.*, looked at cytokines in the serum samples predating RA diagnosis[21]. In addition, they looked at the titer of antibodies to 16 different citrullinated peptide antigens. ACPA were detected as early as 4,000 days (nearly 11 years) before diagnosis. While no one ACPA to a particular peptide antigen was the first antibody in all specimens, antibodies to citrullinated fibrinogen, vimentin, and α-enolase were the earliest responses seen in approximately 30 to 40% of all cases (Figure 3). The number of ACPA and their titer increased in the three years prior to diagnosis. The titer of inflammatory cytokines such as interferon gamma, IL-12, IL-15, IL-6 and TNF-alpha, increased with time, following the rise in ACPA titer and the spreading of ACPA specificities. Deane, *et al.*, came to similar conclusions testing the serum from the military cohort for levels of 14 different cytokines and chemokines. Again, rising numbers of autoantibodies and inflammatory biomarkers predicted the onset of RA. They made the additional important observation that for a given interval between biomarker elevation and disease, the number of elevated biomarkers was less in younger patients suggesting that they were somehow more predisposed to RA development.

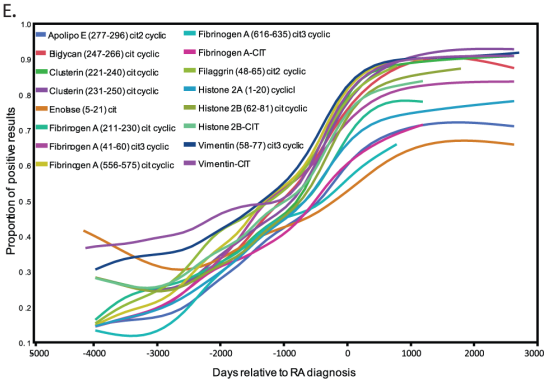


Figure 3. Prevalence of antibodies to various citrullinated peptides before RA diagnosis. Reproduced from Sokolove, *et al.*

Study of the natural history of lupus prior to complete disease classification has been facilitated by the fact that clinical symptoms appear over time. This is made it possible to

identify persons at risk for development of lupus who may have one or two features of the disease. These patients have been termed Incomplete Lupus Erythematosus, or ILE. A cohort of patients with ILE has been followed at UT Southwestern[22,23]. Typically, these patients have an ANA and one or two other features of SLE. 22 patients were seen who had ILE were seen at baseline and at follow up visits an average of 2.4 years apart (range 0.5-6.5 yr.). In this time period, three of 22 patients progressed to SLE. One of the tools used to follow patients was an autoantigen microarray consisting of over 100 antigens previously described as targets of antibodies in lupus and other autoimmune conditions. The patients progressed from ILE to SLE had higher baseline levels of seven IgG autoantibodies. These antibodies were not necessarily ones commonly associated with SLE or available clinically. They included thyroid peroxidase, thyroglobulin, PCNA, hemocyanin, beta-2 microglobulin, threonine tRNA synthetase and liver cytosol. Levels of some of these autoantibodies were also significantly elevated in the final blood samples of subjects who progressed to SLE. Moreover, the total IgG autoreactivity increased significantly in the subjects who progressed to lupus.

In addition to autoantibodies, several other biomarkers were tested. In established SLE, there is up-regulation of genes driven by Type I interferons (e.g., interferon alpha or beta). The source of this cytokine is presumed to be plasmacytoid dendritic cells chronically simulated by immune complexes and resulting in perpetuation of innate and adaptive autoimmunity. As a group, subjects with ILE also had levels of mRNA for interferon alfa responsive genes that were intermediate between healthy controls and patients with SLE, suggesting that the increased level of autoantibodies was functionally relevant[24]. The ILE subjects also had intermediate levels of certain cytokines including MCP-1, EGF, VEGF, and eotaxin. By combining age, female gender, anti-nuclear antibody titer and overall autoantibody burden, the investigators were able to develop a scoring system that was highly predictive of the progression from ILE to SLE.

Blood relatives of lupus patients also represent an at-risk population for the development of SLE. This is most obvious in twin studies where the concordance rate for monozygotic twins developing lupus is between 24 and 69%, while the concordance for dizygotic twins is between 2 and 5%. Researchers at OMRF have followed 409 unaffected blood relatives of lupus patients (M. Monroe, unpublished). They were evaluated clinically at baseline and at an average of 6.4 years later. Blood was obtained for autoantibody and biomarker analysis. 45 blood relatives of lupus patients transitioned to SLE during this time. Subjects who transitioned had more autoantibodies present at baseline, particularly DNA and RNA binding antibodies. In addition they had higher levels of some biomarkers, Including stem cell factor, MCP-1, MCP-3, and B lymphocyte stimulator (BLyS). They also have lower levels of anti-inflammatory cytokines including IL-10 and TGF-beta. Investigators also administered the SLE-specific portions of a validated, patient self-reported questionnaire, termed the Connective Tissue Disease Screening Questionnaire" (CSQ). This questionnaire is designed to elicit symptoms of lupus and other connective tissue diseases from patients. It has shown to have high validity and sufficient sensitivity and specificity to serve as an initial screening tool in high-risk populations. Using generalized estimating equations, a model that includes the number of SLE classification criteria at baseline, the baseline CSQ, and baseline concentrations of stem cell factor and TGF-beta, but not age, gender, race or

autoantibody status was able to correctly classify 89% of blood relatives of SLE patients who would later go on to develop lupus.

Practice Point #1

- The "pre-clinical" phase of autoimmune diseases exists 5-10 years before the onset of symptoms
- This phase is characterized by accumulation of multiple autoantibodies as well as immune activation.
  - Different epitopes of the same antigen
  - Different and unrelated antigens
- During this time, there is the gradual onset of typical symptoms such as arthralgia/arthritis or rash

These studies and many others support the current paradigm (Figure 4) for the development of autoimmune rheumatic diseases[25,26]. The development of autoantibodies, in and of themselves, may not be pathogenic. Their presence is influenced by the genetic makeup of the person, as well as environmental factors such as infections, exposure to UV light, tobacco smoke, nutritional factors, hormones, and the person's microbiome. These people are felt to have "benign autoimmunity". That is, they have evidence of serological autoimmunity without evidence of tissue damage. In a subset of these persons, the number and titer of autoantibodies increases, triggering activation of the inmate and adaptive immune systems. The triggers for such epitope spreading and expansion of autoantibody repertoires are not known. Ultimately, the level of inflammatory mediators and immune complexes reaches a critical threshold and damage occurs to tissues and cells. Unfortunately, treatment of the patient at this point is centered on limiting tissue damage. If we are to prevent autoimmune disease, treatment must be given to people who do not have signs or symptoms of disease. The question is how to screen and whom to treat.

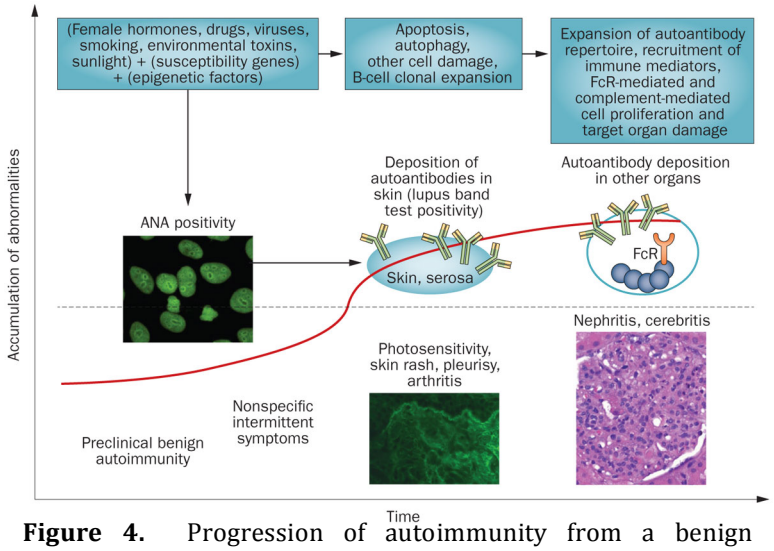


Figure 4. Progression of autoimmunity from a benign preclinical phase characterized by one or a few antibodies to an intermediary phase with epitope spreading, immune activation and early symptoms followed by immune complex damage to tissues. Reproduced from Olsen, NJ and Karp DR.

Screening for Rheumatoid Arthritis

It is tempting to use the autoantibodies that are part of the disease classification criteria for screening. These include rheumatoid factor, anti-CCP, and anti-nuclear antibody (ANA).

However, this is very inefficient and inaccurate due to both the sensitivity and specificity of the tests and the prevalence of RA and SLE in the general population. The best predictive value is probably from the tests for RA. A combination of anti-CCP and two or more isotypes of RF had 74% sensitivity and 98.6% specificity for the development of future RA in the military cohort compared to matched military controls[27]. Using data from Dutch blood donors, Neilen, *et al.*, the risk of developing RA in a five year follow up period was estimated to be 100% for people who had both anti-CCP and IgM-RF[17]. Having either antibody alone lessened the positive predictive value, particularly when applied to the general population versus a "high-risk" population defined as having two or more first-degree relatives with RA. However, other cohorts have yielded a much lower estimate (16%) of the positive predictive value of RF and anti-CCP in the general population[16].

	Blood Donor Population		5-Year Risk of Developing RA	
	Sensitivity	**Specificity**	**General pop.**	**High-risk pop.**
IgM -RF	20.5%	98.6%	1.5%	37.7
Anti-CCP	28.9	99.5	5.3	69.4
IgM-RF or anti-CCP	36.5	98.1	1.9	43.8
IgM-RF and anti-CCP	13.0	100	100	100

A few studies have assessed the utility of anti-CCP or RF in a prospective manner. Dutch researchers studied 147 patients who had arthralgia, but not arthritis[28]. The median duration of symptoms was 12 months, and they were followed for a median of 28 months after enrollment in the study. At the beginning of the study, 52 subjects were IgM-RF positive, 50 had anti-CCP, and 45 had both antibodies. 29 (~20%) of the arthralgia patients developed RA during the period of observation. Anti-CCP was the strongest predictor of arthritis development with a hazard ratio of 6.0 (1.8-19.8). IgM-RF was not an independent predictor of RA development, but added to the risk of anti-CCP.

Investigators from Harvard and Sweden have used the Nurses Health Study (NHS) as well as early RA cohorts to develop a model that predicts RA development without serology[29]. Using a combination of 39 genetic traits along with environmental features such as age, tobacco use, alcohol use, occupation, hormonal effects (e.g., menarche, parity, breastfeeding, estrogen use), and educational level. Their model was able to correctly classify 71.6% of women in the NHS and early RA cohorts, and 75.6% of men with early RA. The authors note that this study was performed on mainly Caucasian populations, and further work is needed to validate the model in prospective studies. It seems likely that a screening test for an RA prevention trial should include genetic factors, environmental factors, as well as autoantibody status.

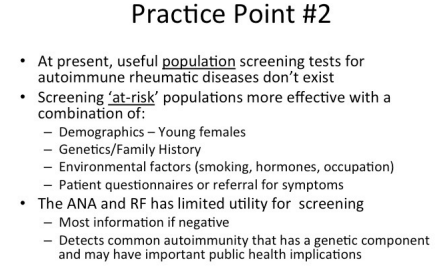
The (Dis)Utility of the ANA to Screen for Lupus

The use of ANA to screen for lupus is even more problematic. This is due to the high rate of ANA positivity in the general population. Classically, anti-nuclear antibodies are detected by indirect immunofluorescence of the patient's serum applied to a cultured carcinoma cell line. This method is reported positive if the laboratory technician detects fluorescence of the cell nuclei following the application of fluorochrome-labeled anti-human IgG. The

degree of positivity is typically reported as a reciprocal titer. Using this method, ANA are seen in approximately 25% of serum samples from healthy individuals at a dilution of 1:40[30]. 5% of healthy controls will have an ANA at a dilution of 1:160. For purely statistical purposes, this is typically the cut off for the upper limit of normal. In a study of 4,754 subjects enrolled in the NHANES survey, an overall ANA positivity of 13.8% at 1:80 dilution of serum was seen[31]. This translates to over 30 million Americans with a positive ANA. Women and non-Hispanic Blacks were more likely to have a positive result. Since the ANA by its nature can detect many nuclear antigens, they tested several known antigenic specificities. The most common antibodies in the general population were anti-Ro (an RNA binding protein, 3.9%) and anti-Su (a microRNA processing protein, 2.4%).

These data are similar to a recent study by Wakeland and colleagues at UT Southwestern (P. Raj, unpublished). They have studied ANA titers in 2,223 healthy individuals using an ELISA based assay that allows measurement of antibodies to nuclear antigens as a continuous variable. As before, approximately 25% of subjects had a value above the cut-off defined by the manufacturer as "elevated". However, the distribution of ANA values appears to be the result of several normally distributed populations. The largest is the group with very low values classified as "negative". Approximately 18% of subjects were in a moderately high group and 7% were in a very high distribution. This distribution consisted only of females, confirming previous observations. Using the auto-antigen array, the breadth of autoreactivity in the ANA+ individuals was shown to be quite significant with the majority of subjects making antibodies to both nuclear and non-nuclear antigens. Nearly 40% of control subjects made antibodies to more than five self antigens. This study was undertaken with control subjects for SLE genotypic studies. Not surprisingly, ANA positivity has similar genetics to SLE with a strong signal in the human major histocompatibility complex. This reinforces the idea that benign autoimmunity is genetically determined and is not the limiting step in the progression to clinical autoimmunity.

Recent data from Solow and colleagues at UT Southwestern suggest that even the low levels of ANA seen in the healthy population may be indicators of clinically important immune-mediated events. As part of a prior analysis of ANA in the general population[23], this test was performed on participants in the Dallas Heart Study (DHS), a longitudinal cohort of Dallas County residents with in-depth study of their cardiovascular disease risk factors. As expected, approximately one-quarter of the DHS study population had an elevated ANA. What is unexpected is that ANA is correlated with cardiovascular death and all-cause mortality. It is known that established SLE and RA are associated with excess cardiovascular disease, even when controlled for traditional risk factors[32], and high titers of autoantibodies have been associated with cardiovascular disease[33,34]. The DHS data show that this extends to the subjects in the general population who neither have, or are suspected of having, SLE, and to much lower levels of ANA than previously studied. Weak associations were seen between ANA and markers of endothelial activation



and inflammation. This suggests that while ANA are often ignored in a person who lacks clinical features of autoimmune disease, that person may still be at risk for other conditions, such as premature cardiovascular disease. Since these levels of ANA are seen in tens of millions of Americans, this finding may truly represent a public health burden.

Preventing Autoimmune Rheumatic Diseases

Can RA and SLE be prevented? The large body of evidence documenting several years of "benign autoimmunity" before the onset of symptoms suggests that a window of opportunity exists when immunomodulation can delay the onset of disease or keep it from occurring entirely. There is some evidence supporting this concept in RA[35]. In several double-blind, placebo-controlled trials, methotrexate[36], abatacept[37], and glucocorticoids[38] have been shown to delay the onset of RA in subjects who had evidence of inflammatory arthritis, but who could not be classified with definite disease. These studies have been of short duration (one year or less) and the differences between placebo and treatment small (15-20% less RA in the treatment groups). By their design, they looked at early patients – 1-2 joints with inflammatory arthritis – not pre-clinical disease.

Investigators at the University of Colorado have begun a multi-center trial, Strategy to Prevent Rheumatoid Arthritis (StopRA) through the Autoimmunity Centers of Excellence, a program supported by the National Institute of Allergy and Infectious Diseases. They will use a panel of biomarkers to identify subjects with serological autoimmunity before the onset of inflammatory arthritis. They will then be randomized to receive either placebo or hydroxychloroquine (HCQ). HCQ has a number of properties that make it attractive as an agent to prevent autoimmune disease[39,40]. It has a low toxicity, making it acceptable to people with few or no symptoms. HCQ is already approved by the FDA for use in established RA and SLE, although the current use in RA is typically limited to combination therapy with agents such as methotrexate.

In SLE, HCQ has been shown to delay onset of disease flares[41,42], limit organ damage[43-45], and increase survival[46,47]. In clinical practice, rheumatologists commonly give HCQ to ANA-positive patients who do not meet criteria for SLE, because, "it couldn't hurt." However, there is no prospective, and little retrospective data to support this. For example, twenty-six of the 130 US military personnel who developed SLE were given HCQ prior to their diagnosis[48]. These patients were less likely to have proteinuria and lymphopenia than the patients who were not treated, but had similar prevalence of other clinical features. Overall, patients who received HCQ at or near the time of their first symptom had a median time to SLE classification that was much greater than those that did not get HCQ (1.08 vs. 0.29 yr., p=0.018). The total number of autoantibodies seen in the HCQ group was less than in the un-treated group, although there were no significant differences in the prevalence of any individual autoantibody owing to the small sample size.

Based on these data, a multi-center clinical trial led by investigators at UT Southwestern and Penn State Hershey Medical Center has been planned. This trial will follow subjects who are ANA positive and have one other clinical or laboratory feature of SLE. They will be assigned to HCQ or placebo and followed for two years. The primary endpoint will be the

number of SLE criteria fulfilled at the end of the trial. Secondary endpoints will be the diagnosis of other autoimmune rheumatic diseases such as Sjögren's syndrome, need for other therapies, and a series of biomarkers including autoantibodies, genomics, gene expression, and cytokine levels. If successful, this study will provide much needed information on the natural history of SLE, establish the true efficacy for a commonly used but unproven therapy, and set the stage for other primary prevention trials in lupus.

Conclusion

The prevention of autoimmune rheumatic diseases would have a large beneficial impact on human health. However, the appropriate screening tools and prevention strategies are not established. Many retrospective and a few prospective studies have clearly shown that increased levels of autoantibodies and markers of inflammation occur years before the onset of symptoms. No one marker has sufficient sensitivity and specificity to accurately populations that can be targeted for primary prevention strategies, and combinations of markers, including genetics, environmental risks, and immunological markers will be necessary. Efforts to prevent diseases such as RA or SLE are in early stages. Proof-of-concept studies have shown promise in the delay of progression from early stage to established disease. What are needed are studies that lead to cessation of disease progression in people in the pre-clinical phase.

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Appendix

Classification Criteria for RA[5]

Must have ≥ 6 points		Score
Joint Involvement*		
	1 large joint	0
	2-10 large joints	1
	1-3 small joints	2
	4-10 small joints	3
	>10 joints	5
Serology		
	Negative RF **AND** negative ACPA	0
	Low positive RF **OR** ACPA	2
	High positive RF **OR** ACPA	3
Chronicity		
	Symptoms < 6 weeks	0
	Symptoms ≥ 6 weeks	1
Acute Phase Reactants		
	Normal ESR and/or CRP	0
	Elevated ESR and/or CRP	1

***Large joints – Shoulders, elbows, hips, knees, ankles; Small joints – wrists, thumb IP, MCP, PIP, 2-5 MTP.**

Classification Criteria for SLE[6]

Clinical Features
Acute cutaneous lupus (malar rash, photosensitive rash, and others)
Chronic cutaneous lupus (discoid LE and others)
Oral/nasal ulcers
Non-scarring alopecia
Synovitis in ≥2 joints
Serositis – Pleural or pericardial
Urine protein-to-creatinine ratio ≥0.5 or red cell casts
Neurologic features (seizures, psychosis, mononeuritis, myelitis, cranial neuropathy, and acute confusional state)
Hemolytic anemia
Leukopenia < 4,000/mm^3 or lymphopenia < 1,000/mm^3
Thrombocytopenia < 100,000/mm^3
Immunological Features
Anti-nuclear antibody
Anti-dsDNA
Anti-Smith (Sm)
Anti-phospholipid antibody testing Lupus anticoagulant False-positive RPR Medium/High anti-cardiolipin Medium/High anti-β2 glycoprotein I
Low complement C3, C4, or CH50
Direct anti-globulin test without hemolysis

Classification requires at least 4 features be present, including at least one of six immunological features and one of 11 clinical features. Alternatively, the patient must have lupus nephritis by biopsy plus ANAs, anti-DNA or both.