Rapid review

Autoantibodies as predictors of disease

R Hal Scofield

Context Many human diseases are the result of autoimmune attack, presumably related to a loss of tolerance to self. Autoimmune disease can be divided into either organ-specific illnesses, such as thyroid disease, type 1 diabetes, and mysasthenia gravis, or systemic illnesses, such as rheumatoid arthritis and systemic lupus erythematosus. The pathogenesis of autoimmune damage also segregates autoimmune disease in that some diseases or manifestations are mainly induced by autoantibodies. Pathogenesis may be mainly mediated by autoimmune T lymphocytes. Notwithstanding the underlying mechanism of disease, virtually all autoimmune diseases are associated with circulating autoantibodies, which bind self-protein. Furthermore, for many diseases these autoantibodies are found in serum samples many years before disease onset.

Starting point In the past several years a new autoantibody specificity has been identified in the sera of patients with rheumatoid arthritis. These autoantibodies bind citrulline, a post-translational modification of arginine. Markus Nielen and colleagues recently studied the presence of these autoantibodies and rheumatoid factor in blood donors who later developed rheumatoid arthritis (*Arthritis Rheum* 2004; **50:** 380–86). About half the patients were positive for at least autoantibody at a median of 4·5 years before the onset of disease. The negative predictive value of these tests was high, while the positive predictive value was very high.

Where next? Autoantibodies might not be directly responsible for many of the manifestations of autoimmune disease, but they are markers of future disease in presently healthy individuals. Long-term large studies of outcome are needed to assess the use of assaying autoantibodies for prediction of disease. Such data could lead to intervention trials to prevent autoimmune disease, as are already underway in type 1 diabetes.

Lancet 2004; **363:** 1544–46

Arthritis and Immunology Program, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104, USA; Department of Medicine, University of Oklahoma Health Sciences Center; and Department of Veterans Affairs Medical Center, Oklahoma City
(Prof R Hal Scofield MD)

(e-mail: hal-scofield@omrf.ouhsc.edu)

Although the incidence and prevalence of individual autoimmune diseases are not high (table), the population burden of the disease is large and underestimated.[1] Serum autoantibodies, which appear long before onset of clinical disease, are a characteristic feature, as recently emphasised by Markus Nielen and colleagues.[2] Studying patients with rheumatoid arthritis, these researchers found antibodies for IgM rheumatoid factor or anticyclic-citrullinated-peptide (anti-CCP) in serum samples taken a median of 4·5 (range 0·1–13·8) years before disease onset. The negative predictive value of these tests was 75% and the positive predictive value was even higher (100%). Thus the presence of autoantibodies in otherwise healthy individuals might be a marker of future autoimmune disease or, alternatively, certain autoantibodies might predict the course in a person with established disease.

Autoantibodies in rheumatoid arthritis

In another recent study of individuals with stored serum who went on to develop rheumatoid arthritis, Rantapää-Dahlqvist et al[3] showed the predictive ability of anti-CCP, which is highly sensitive for rheumatoid arthritis. In a population-based survey of over 150 000 people in northern Sweden, these investigators found 83 who went on to be diagnosed with rheumatoid arthritis. Anti-CCP was found in a third of these 83, on average several years before onset of disease. The negative predictive value of this test was high, and the positive predictive value in a population with joint symptoms was 62%.

Autoantibodies in pregnancy

Autoantibodies associated with rheumatoid arthritis may also predict disease in healthy individuals in whom hormonal changes might be a trigger for autoimmune disease. In 401 healthy pregnant women followed up for a year, none with a negative rheumatoid factor developed rheumatoid arthritis, whereas two of nine with rheumatoid factor developed the disease.[4] Similar data have been seen for postpartum autoimmune thyroid disease.[5] In 1594 consecutive pregnant women, antithyroid-peroxidase antibodies (anti-TPO) were found in 83 (5·2%) when studied within 3 days of delivery, and autoimmune thyroid disease developed in 38 (2·4%) in the year of follow-up.[5] Of the 38 who developed clinical disease, 37 had a titre over 1:1600, whereas of the 33 not developing clinical disease, 30 had a titre under 1:400. Anti-TPO at 1:1600 or higher at delivery had a 97% sensitivity and a 91% specificity for postpartum autoimmune thyroid disease. In another study,[6] 291 pregnant women were tested for anti-TPO at 12 and 32 weeks' gestation and 4 weeks' postpartum. 15 developed autoimmune thyroid disease in the first year postpartum, and ten of these had anti-TPO. Again, the titre of anti-TPO was lower in those that did not develop clinical disease.

The prediction of type 1 diabetes after gestational diabetes by diabetes-associated antibodies at the time of delivery has been studied.[7] 184 women with gestational diabetes were studied for HLA, antiglutamic-acid-decarboxylase (GAD) and antityrosine-phosphatase-like protein,

and islet-cell autoantibodies, and followed up for 5 years. 43 were positive for at least one diabetes-associated autoantibody, and 24 of these developed type 1 diabetes. A combination of HLA typing and autoantibody measurement was highly predictive of future type 1 diabetes. Thus, in pregnancy, future autoimmune diseases can be predicted with autoantibody assay at delivery. Of course, the postpartum woman might be particularly at risk for autoimmune disease. So whether or not such testing can be done in a general population with high positive and negative predictive values is not known.

Another pregnancy-related condition has been studied. Mothers with the autoantibodies associated with systemic lupus erythematosus and Sjögren's syndrome, anti-Ro (or SSA) and anti-La (or SSB), can give birth to babies with neonatal lupus, which is caused by maternal antibody that crosses the placenta. Most mothers of these babies are healthy at the time of delivery, but several studies have done long-term follow-up. The initial study by McCune et al[8] followed up 21 mothers for an average of 4·5 years after birth of a baby with neonatal lupus. Eight of the 11 who were initially asymptomatic became symptomatic in follow-up, mostly with dry eyes, compared with only one of 37 control mothers. With objective testing of keratoconjunctivitis sicca, another study[9] found that 14 mothers asymptomatic at delivery of a baby with congenital heart block had at least one abnormal test at a mean 10·6 years from delivery. Waltuck and Buyon[10] found that of 22 healthy mothers, 11 remained well, three developed Sjögren's syndrome, three developed systemic lupus erythematosus, and seven developed an undifferentiated syndrome. So these mothers who have babies with neonatal lupus and anti-Ro/La in their serum subsequently commonly develop symptoms consistent with Sjögren's syndrome, which is strongly associated with the simultaneous finding of anti-Ro and anti-La.

Autoantibodies in other diseases

Persons serving in the US military have blood drawn and serum stored in the US Armed Services Serum Repository about every 2 years, and the Repository has about 30 million samples from 6 million individuals. My colleagues and I identified 132 military personnel diagnosed with systemic lupus erythematosus who had at least one sample in the Repository before the diagnosis, and two matched controls for each future patient with systemic lupus erythematosus. Antinuclear antibodies and anti-Ro appeared as early as 10 years before first onset of disease, with 47% having anti-Ro and 78% having antinuclear antibodies before onset. Thus studies of anti-Ro/La-positive mothers giving birth to babies with neonatal lupus may be of too short a duration. Antibodies binding native (or double-stranded) DNA appeared about 2·5 years before diagnosis in 55% of the cohort, whereas antibodies against nuclear ribonuclear protein and anti-Sm appeared only a few months before disease. Almost no new antibodies appeared after the onset of clinical disease Furthermore, a few controls had antinuclear antibodies or anti-Ro but no other specific antibody.[11] Thus the presence of lupus-associated antibodies was both sensitive and specific for the subsequent occurrence of systemic lupus erythematosus.

Primary biliary cirrhosis is associated with antimitochondrial antibodies, and the main target of these autoantibodies is the E2 component of the pyruvate dehydrogenase complex (PDC). Kisand and colleagues[12] studied antibodies to recombinant E2-PDC in 1461 individuals and antibodies to native purified PDC in a subset of 497. Eight asymptomatic individuals with at least one of these autoantibodies were followed up for 9 years.

Organ-specific diseases	Known antigens
Addison's disease	21-hydroxylase
Coeliac disease	Tissue transglutaminase
Type 1 diabetes	GAD-65, insulin, IA-2A
Graves' hyperthyroidism	Thyroid-stimulating-hormone receptor
Hashimoto's thyroiditis	Thyroid peroxidase, thyroglobulin
Myasthenia gravis	Acetylcholine receptor
Goodpasture's syndrome	Type IV collagen
Pemphigus vulgaris	Desmoglein 3
Pernicious anaemia	H/KATPase, intrinsic factor
Primary biliary cirrhosis	E2 PDC
Vitiligo	Tyrosinase, SOX-10
Multiple sclerosis	Myelin basic protein, myelin oligodendritic glycoprotein
Systemic diseases	
Systemic lupus erythematosus	Spliceosomal snRNP, Ro/La (SSA/SSB) particle, histone and native DNA
Sjögren's syndrome	Ro/La ribonuclear particle, muscarinic receptor
Rheumatoid arthritis	Citrillunated cyclic peptide, IgM
Dermatomyositis/polymyositis	t-RNA synthetases
Diffuse systemic sclerosis	Topoisomerase
Limited systemic sclerosis (CREST)	Centromere proteins

CREST=calcinosis, Raynaud's phenomenon, oesophageal dysmotility, sclerodactyly, and telangiectasia.

Selected autoimmune diseases and autoantigens

Three developed abnormal liver-enzyme tests and antimitochondrial antibodies by immunofluorescence. The five who did not develop liver-enzyme abnormalities had lower anti-E2-PDC titres, only one immunoglobulin class binding E2-PDC, and antibodies that did not inhibit enzyme activities. Antibody-negative individuals were not followed up.

In Addison's disease, use of autoantibodies, both anti-adrenal-cortical (ACA) and anti-21-hydroxylase antibodies, has been studied.[13] Organ-specific autoimmune diseases occur together more than expected by chance, so patients with one disease are at higher risk for another autoimmune disease. In the initial study from 1983, one of 284 healthy individuals had ACA and this individual went on to develop Addison's disease. Meanwhile, eight of 1045 individuals with other organ-specific autoimmune disease had these antibodies, of which five had normal adrenal function. In a mean follow-up of 20 months, four of these five developed overt Addison's disease.[14] One study[15] found ACA in 15 of 2543 healthy individuals, and these 15 had higher concentrations of adrenocorticotropic hormone, which indicates compensated mild hypoadrenalism. In 808 children with organ-specific autoimmune disease, 14 (1·7%) had ACA. Long-term follow-up of ten of these children showed development of overt Addison's disease in nine. The remaining children had compensated subclinical hypoadrenalism. None of ten controls without ACA or anti-21-hydroxylase developed Addison'sdisease.[16] However, the rate of progression to overt Addison's disease was lower in adults.[17] Among 8840 adults, 67 had ACA. Follow-up of 48 ACA-positive and 20 matched ACA-negative individuals showed half of the ACA-positive individuals remained normal (mean follow-up 45 months). Ten of the ACA-positive individuals progressed to overt Addison's disease and seven developed compensated hypoadrenalism.[17] As in other diseases, the titre of Addison's-associated autoantibodies is correlated to progression.[18]

There are reports of remission with treatment in patients with adrenal autoantibodies.[19,20] Three patients with Graves' disease and severe ophthalmopathy as well as adrenal autoantibodies and mildly impaired adrenal function were treated with high doses of glucocorticoids for the eye disease. In 5 years of follow-up, 6 months of therapy resulted in a loss of the adrenal autoantibodies and a return of normal adrenal function. Meanwhile, in 11 controls with

adrenal autoantibodies and similarly mildly impaired adrenal function (increased plasma renin activity, decreased aldosterone, and decreased cortisol response to synthetic adrenocorticotropic hormone), two progressed to overt Addison's disease and eight showed worsened adrenal function.[19] This study was not a randomised trial, but the data suggest that incipient adrenal failure and adrenal autoimmunity can be halted.

Autoantibodies in diabetes

While progress about the prediction of future disease by autoantibodies in the examples above is substantial, this progress pales compared with that made in type 1 diabetes mellitus.[21] Many studies have assessed GAD, anti-insulin, anti-islet-cell, and/or anti-IA-2A in non-diabetic siblings of patients with autoimmune diabetes. The presence of two or more of these autoantibodies is highly correlated with progression to overt diabetes. The prediction of diabetes has moved into the general population.[22] 12 of 4502 children (median age 14 years) had more than one diabetes-associated autoantibody, and six developed type 1 diabetes at an 8-year follow-up. No child with one or no autoantibodies had diabetes. So the presence of two autoantibodies was over 99·5% specific and gave a 50% positive predictive value. In Finland,[23] a population-based, birth-to-age-four screening programme that combined HLA typing and autoantibodies in 31 526 babies identified 75% of those developing diabetes. An immunological screening strategy in which only those with increased risk on the basis of HLA typing might be less expensive.[24] These data[24] indicate that population-based screening for diabetes is possible and effective. Several large-scale trials are underway in which individuals with two or more diabetes-associated autoantibodies receive immune-modulating therapy, such as nasal insulin in the Type 1 Diabetes Prediction and Prevention Project in Finland.[23]

Conclusions

Thus the data indicate that autoimmune disease is preceded by a long preclinical phase in which individuals can be identified by the presence of autoantibodies. Such identification might allow immunological treatment whereby disease is prevented, as is already being studied in diabetes. Alternatively, even if disease cannot be prevented, perhaps life-threatening but treatable conditions could be avoided, such as thyroid storm and Addisonian crisis. For all the diseases, except type 1 diabetes, further work is needed to assess the natural history of disease progression and predictive value of autoantibodies in healthy individuals. Predictive values will vary with pre-test probabilities, which will be greatly different in autoimmune diseases with widely varying prevalences. Thus testing and follow-up of special populations, such as pregnant women or those with one organ-specific autoimmune disease, probably cannot be extrapolated to the general population, which is at lower risk of disease.

Encouragingly, high-throughput protein-array techniques are already available in which hundreds of autoantibody specificities can be analysed simultaneously.[25] This technology might allow prospective studies of large numbers of healthy individuals for the development of many autoimmune diseases. Another alternative is to apply this technology to existing databases, such as the US Armed Forces Serum Repository.[11] Testing these samples for multiple autoantibodies with protein arrays and correlating the results with diagnosed diseases might answer several important questions about the preclinical natural history of autoimmune diseases. Once natural history is established and predictive values of autoantibodies known, intervention trials in diseases other than diabetes will be ready to start.

I have no conflict of interest to declare.

References

1 Jacobson DL, Gange SJ, Rose NR, Graham NM. Epidemiology and estimated population burden of selected autoimmune diseases in the United States. *Clin Immunol Immunopathol* 1997; **84:** 223–43.

2 Nielen MM, van Schaardenburg D, Reesink HW, et al. Specific autoantibodies precede the symptoms of rheumatoid arthritis: a study of serial measurements in blood donors. *Arthritis Rheum* 2004; **50:** 380–86.

3 Rantapää-Dahlqvist S, de Jong BAW, et al. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. *Arthritis Rheum* 2003; **48:** 2741–49.

4 Iijima T, Tada H, Hidaka Y, et al. Prediction of postpartum onset of rheumatoid arthritis. *Ann Rheum Dis* 1998; **57:** 460–63.

5 Kita M, Goulis DG, Avramides A. Post-partum thyroiditis in a mediterranean population. *J Endocrinol Invest* 2002; **25:** 513–19.

6 Kuijpens JL, De Hann-Meulman M, Vader HL, et al. Cell-mediated immunity and postpartum thyroid dysfunction. *J Clin Endocrinol Metab* 1998; **83:** 1959–66.

7 Ferber KM, Keller E, Albert ED, Ziegler AG. Predictive value of human leukocyte antigen class II typing for the development of islet autoantibodies and insulin-dependent diabetes postpartum in women with gestational diabetes. *J Clin Endocrinol Metab* 1999; **84:** 2342–48.

8 McCune AB, Weston WL, Lee LA. Maternal and fetal outcome in neonatal lupus erythematosus. *Ann Intern Med* 1987; **106:** 518–23.

9 Julkunen H, Eronen M. Long-term outcome of mothers of children with isolated heart block in Finland. *Arthritis Rheum* 2001; **44:** 647–52.

10 Waltuck J, Buyon JP. Autoantibody-associated congenital heart block: outcome in mothers and children. *Ann Intern Med* 1994; **120:** 544–51.

11 Arbuckle MR, McClain MT, Rubertsen M, et al. Autoantibodies are present years before the clinical onset of systemic lupus erythematosus. *N Engl J Med* 2003; **349:** 1526–33.

12 Kisand KE, Metskula K, Kisand KV, Kivik T, Gershwin ME, Uibo R. The follow-up of asymptomatic persons with antibodies to pyruvate dehydrogenase in adult population samples. *J Gastroenterol* 2001; **36:** 248–54.

13 Betterle C, Dal Pra C, Mantero F, Zanchetta R. Autoimmune adrenal insufficiency and autoimmune polyendocrine syndromes: autoantibodies, autoantigens, and their applicability in diagnosis and disease prediction. *Endocr Rev* 2002; **23:** 327–64.

14 Betterle C, Zanette F, Zanchetta R, et al. Complement-fixing adrenal autoantibodies as a marker for predicting onset of idiopathic Addison's disease. *Lancet* 1983; **1:** 1238–41.

15 Ketchum CH, Riley WJ, Maclaren NK. Adrenal dysfunction in asymptomatic patients with adrenocortical autoantibodies. *J Clin Endocrinol Metab* 1984; **58:** 1166–70.

16 Betterle C, Volpato M, Rees Smith B, et al. II: Adrenal cortex and steroid 21-hydroxylase autoantibodies in children with organ-specific autoimmune diseases. *J Clin Endocrinol Metab* 1997; **82:** 939–42.

17 Betterle C, Volpato M, Rees Smith B, et al. I: Adrenal cortex and steroid 21-hydroxylase autoantibodies in adult patients with organ-specific autoimmune diseases. *J Clin Endocrinol Metab* 1997; **82:** 932–38.

18 Laureti S, De Bellis A, Muccitelli VI, et al. Levels of adrenocortical autoantibodies correlate with the degree of adrenal dysfunction in subjects with preclinical Addison's disease. *J Clin Endocrinol Metab* 1998; **83:** 3507–11.

19 De Bellis A, Bizzarro A, Rossi R, et al. Remission of subclinical adrenocortical failure in subjects with adrenal autoantibodies. *J Clin Endocrinol Met* 1993; **76:** 1002–07

20 De Bellis A, Falorni A, Laureti S, et al. Time course of 21-hydroxylase antibodies and long-term remission of subclinical autoimmune adrenalitis after corticosteroid therapy: case report. *J Clin Endocrinol Metab* 2001; **86:** 675–78.

21 Notkins AL, Lernmark A. Autoimmune type 1 diabetes: resolved and unresolved issues. *J Clin Invest* 2001; **108:** 1247–52.

22 LaGasse JM, Brantley MS, Leech NJ, et al. Washington State Diabetes Prediction Study. Successful prospective prediction of type 1 diabetes in schoolchildren through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes Prediction Study. *Diabetes Care* 2002; **25:** 505–11.

23 Kupila A, Muona P, Simell T, et al. Juvenile Diabetes Research Foundation Centre for the Prevention of Type I Diabetes in Finland. Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. *Diabetologia* 2001; **44:** 290–97.

24 Hahl J, Simell T, Ilonen J, Knip M, Simell O. Costs of predicting IDDM. *Diabetologia* 1998; **41:** 79–85.

25 Robinson WH, DiGennaro C, Hueber W, et al. Autoantigen microarrays for multiplex characterization of autoantibody responses. *Nat Med* 2002; **8:** 295–301.