Predicting and Preventing Autoimmunity, Myth or Reality?

MICHAL HAREL[a] AND YEHUDA SHOENFELD[a,b]

[a]*Center for Autoimmune Diseases, Department of Medicine B, Chaim Sheba Medical Center Tel-Hashomer 52621, Tel Aviv, Israel, and the Sackler Faculty of Medicine, Tel-Aviv University, 69978 Tel-Aviv, Israel*

[b]*Incumbent of the Laura Schwarz-Kipp Chair for Research of Autoimmune Diseases, Tel-Aviv University, 69978 Tel-Aviv, Israel*

ABSTRACT: **Many autoimmune diseases are chronic conditions that progress over the course of years, and are characterized by the presence of autoantibodies that precede the overt disease by months or years. As examples, the presence of two islet cell antibodies (ICA) are associated with a 50% risk of developing diabetes mellitus in 5 years, anticyclic citrullinated (anti-CCP) antibodies are found in the sera of rheumatoid arthritis (RA) patients a median of 4.5 years before the overt disease, and in systemic lupus erythematosus (SLE), patients accrue antibodies throughout a foreseen course during the 3–4 years prior to the clinical symptoms. This ability to predict autoimmune diseases, or rather their clinical manifestations, leads to the prospect of screening healthy individuals for autoantibodies. The importance of such a notion lies not only in the ability to prevent life-threatening manifestations, such as Addisonian's crisis and thyroid storm, but also in the ability to treat and even prevent overt autoimmune diseases. Among such documented treatment modalities are administration of aspirin in antiphospholipid syndrome, ursodeoxycholic acid in primary biliary cirrhosis (PBC), vitamin D in SLE and autoimmune thyroid diseases (AITD), and more. Although additional studies are still needed to fully assess these notions, as well as the appropriate screening strategies to apply them, one cannot ignore the prospect of predicting and preventing autoimmunity.**

KEYWORDS: **autoantibodies; diabetes mellitus; rheumatoid arthritis; systemic lupus erythematosus; antiphospholipid syndrome; primary biliary cirrhosis; autoimmune hepatitis; Crohn's; ulcerative colitis; Addison's; thyroid; pemphigus; miscarriages**

Address for correspondence: Yehuda Shoenfeld, M.D., F.R.C.P., Department of Medicine 'B' and Center for Autoimmune Diseases, Sheba Medical Center, Tel-Aviv University, Tel-Hashomer 52621, Israel. Voice: 972-3-5302652; fax: 972-3-5352855.
e-mail: Shoenfel@sheba.health.gov.il

Ann. N.Y. Acad. Sci. 1069: 322–345 (2006). **© 2006 New York Academy of Sciences.**
doi: 10.1196/annals.1351.031

INTRODUCTION

Taken as a group, autoimmune diseases are the third leading cause of morbidity and mortality in the industrialized world, only surpassed by cancer and heart disease.[1] Many autoimmune diseases are chronic diseases that progress over the course of years and are characterized by the presence of autoantibodies that precede the overt disease.[1] Autoantibodies may also predict specific clinical manifestation, disease severity, and rate of progression,[2] as well as specific clinical phenomena, such as autoimmune pregnancy loss.[3] The identification of these markers and the assessment of their predictive value might enable secondary prevention using specific drugs, such as ursodeoxycholic acid in primary biliary cirrhosis (PBC), or by immunological treatment, as is already being studied in type 1 diabetes mellitus. In addition, the ability to predict the severity of disease and its specific clinical manifestation allows tertiary prevention of disease complications. Such prevention may be accomplished by relatively simple adjustments of therapy and lifestyle including administration of aspirin[4] as well as vitamin D,[5] dietary modifications,[6] and avoidance of ultraviolet light exposure[7] or of oral contraceptives.[8]

The following is a brief article of the predictive role of autoantibodies in various autoimmune diseases and of specific pathologic phenomena associated with autoimmune responses.

TYPE 1 DIABETES MELLITUS

Type 1 diabetes is manifested by a destruction of the pancreatic ß cells that lead to insulin deficiency. At the time of clinical symptoms, 60–80% of the ß cells are destroyed. Insulitis, an inflammatory infiltrate containing large numbers of mononuclear cells and CD8 T cells, typically occurs around or within individual islets.[9]

Islet cell antibodies (ICA) in type 1 diabetes were first discovered in the 1970s and later shown to be antibodies directed against an isoform of glutamic acid decarboxylase (GAD65) and a protein tyrosine phosphatase–like molecule (IA-2). Autoantibodies directed against insulin are found in type 1 diabetes patients as well, but are also developed in patients receiving insulin replacement therapy and thus are not useful for classification of diabetes after such treatment has begun. It has been found that these autoantibodies precede the development of diabetes by many months or years, allowing prediction of overt disease and identification of subjects at high risk of developing diabetes.[9] Prospective studies regarding the predictive value of these antibodies have shown that the presence of autoantibodies directed against two or more antigens is far more strongly associated with the risk of disease than is a high titer of autoantibody to any single antigen (up to a 50% risk of developing type 1 diabetes within 5 years in the presence of both anti-GAD65 and IA-2). Moreover, the combination of

high-risk human leukocyte antigen (HLA) genes with autoantibodies further increases positive prediction.[9]

In addition, the value of diabetes-associated autoantibodies (anti-GAD and IA-2), as well as HLA type in the prediction of type 1 diabetes after gestational diabetes, has been studied. Twenty-four women out of 43 found positive for at least one diabetes-associated antibody developed type 1 diabetes in a 5-year follow-up. A combination of HLA typing and autoantibody measurements has been found highly predictive of future type 1 diabetes in these cases.[10]

The possibility to predict type 1 diabetes resulted in the development of clinical research protocols for the prevention of type 1 diabetes in high-risk patients. These protocols included randomized treatment with either insulin as in the DPT-1 study,[11] or nicotinamide as in the ENDIT study.[12] None of these treatment modalities were able to change the rate of onset of type 1 diabetes compared to placebo. Nonetheless, these studies have contributed greatly to the assessment of factors that control the progression from islet cell autoimmunity to clinical onset, such as baseline glucose tolerance, age, and the number of ICAs detected.[13]

Another application of antibody testing may be the screening of healthy population or population at risk. In Finland, a population-based birth-to-age-4 screening program[14] has combined HLA typing and autoantibodies testing in 31,526 babies, in order to identify patients prior to overt disease. Genetic susceptibility (2.5–15 times the risk of the general population) was determined in all babies through HLA testing. Only those of HLA–DQB1 genotypes *02/*0302 and *0302/x (x not equal to *02, *0301, and *0602) were invited to autoantibody follow-up. Overall, the program has identified about 75% of the children developing diabetes at an early age. Of the 22 children who developed diabetes, 17 were found to carry the risk genotypes. The importance of such a program lies in both the attempt to prevent the overt disease, but also in the prevention of its life-threatening complications, such as diabetic keto-acidosis and coma.

Screening strategy must take into consideration the sensitivity and specificity of any single serological test as well as their combination. Such evaluation was recently performed in the DPT-1 study.[11] It was found that testing for anti-GAD65 and IA-2 achieved a higher sensitivity compared to the testing of any single antibody. Screening for any three antibodies guaranteed detection of all multiple antibody-positive subjects.

Furthermore, a screening program must also take into consideration the validity, sensitivity, and specificity of different laboratory methods regarding the different antibody testing. In the Diabetes Autoantibody Standardization Program, a proficiency evaluation program has shown that in the majority of participating laboratories, GAD and IA-2 antibody assays perform well. It has been possible to identify GAD antibody, IA-2 antibody, and insulin autoantibody (IAA) assays, which achieved high sensitivity and specificity, and to define the characteristics associated with good levels of discrimination between

health and disease. The workshops have shown that good interlaboratory concordance has been achieved if GAD and IA-2 antibody levels are expressed in terms of common WHO units/mL derived from the WHO reference reagent.[15]

In conclusion, the prediction of type 1 diabetes mellitus is now considered feasible by autoantibody testing. In order to achieve significant specificity and sensitivity, multiple-antibody testing, such as anti-GAD and IA-2 would be advised. The combination of such tests with HLA typing may increase sensitivity and specificity. However, its cost and complexity may limit the use of HLA typing as a screening method. As to date, screening of the general population or rather of high-risk population is yet to be justified, mostly on account of the inability to prevent an overt disease. Still, many efforts are being made, and thus further advancement in prevention and treatment of type 1 diabetes mellitus is inevitable.

RHEUMATOID ARTHRITIS

Rheumatoid Arthritis (RA) is a common systemic autoimmune disease with a prevalence of about 1% worldwide. It is marked by a chronic inflammation of the synovial joints that leads to joint swelling, progressive joint erosions, and eventually to disability.[16] The pathogenesis of RA is poorly understood, yet there is evidence of a preclinical or asymptomatic phase of the disease during which there may be already histological evidence of synovitis.[17] Studies have proven that aggressive treatment given early in the course of disease has a great beneficial effect on the outcome. Therefore, early diagnosis prior to joint damage is of great importance.[16]

Several autoantibody systems have been described in association with RA.[16] The autoantibodies most frequently found in patients with RA are antibodies against IgG (IgM rheumatoid factor [IgM-RF]) and antibodies against citrullinated proteins.[17] The latter were originally measured as antibodies against keratin or filaggrin and more recently as anticyclic citrullinated peptide (anti-CCP).[17]

Autoantibodies may be present in RA patients before clinical disease is apparent.[17] It has been shown recently that anti-CCP antibodies are present in the blood of RA patients years before development of overt disease. In a study by Nielen *et al.*,[17] sera of 79 RA patients who had been blood donors, had tested positive for antibodies years before the disease became apparent. About half of the patients were shown to be positive for IgM-RF and/or anti-CCP on at least one occasion before the development of RA symptoms, a median of 4.5 years before symptom onset.

In a similar study by Rantapää-Dahlqvist *et al.*,[18] 83 individuals with RA were identified as having donated blood before presenting with any symptoms of joint disease (median 2.5 years before RA). In samples obtained before the onset of RA, the prevalence of autoantibodies was 33.7% for anti-CCP, 16.9%

for IgG-RF, 19.3% for IgM-RF, and 33.7% for IgA-RF (all highly significant compared with controls). The sensitivities for detecting these autoantibodies >1.5 years and ≤ 1.5 years before the appearance of any RA symptoms were 25% and 52% for anti-CCP, 15% and 30% for IgM-RF, 12% and 27% for IgG-RF, and 29% and 39% for IgA-RF. In conditional logistic regression models, anti-CCP antibody and IgA-RF were found to be significant predictors of RA.[18]

Several other studies have shown the ability of CCP to predict the erosiveness of the developing RA.[16,19] It has been found that combining the anti-CCP test with the routinely used RF test renders the highest prognostic value for RA. These studies concluded that the presence of anti-CCP antibodies can clearly and specifically predict the development of RA, and may indicate progression to an erosive disease.[16]

In conclusion, anti-CCP antibodies and RF may serve as predictive markers of RA and its severity and thus allow early more appropriate management of the disease. Nevertheless, more large-cohort prospective studies are needed in order to further establish the use of these antibodies in routine serological testing for diagnosed RA patients as well as healthy or high-risk individuals.

SYSTEMIC LUPUS ERYTHEMATOSUS

Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease characterized by multisystem involvement in association with autoantibody production. Clinical manifestations of SLE include inflammation and damage to the skin, joints, serosal surfaces, kidneys, and nervous system, often accompanied by fatigue, malaise, and pain.[20]

Antinuclear antibodies directed against nuclear components of the cell are the most characteristic of SLE, although they have limited specificity. Other autoantibodies in SLE include antibodies directed against other molecules, such as phospholipids, cell surface proteins, and humoral factors.[20]

It has been recently found that some of these antibodies are present in the sera of patients long before the clinical manifestation of SLE. In a study by Arbuckle *et al.*,[21] sera of 130 SLE patients were obtained from the Department of Defense Serum Repository. These samples were originally obtained from U.S. Armed Forces personnel on enlistment and, on average, every other year thereafter, all before the diagnosis of SLE. In 88% of these patients, at least one of the autoantibodies tested was present, a mean of 3.3 years before the diagnosis. Antinuclear, antiphospholipid , anti-Ro, and anti-La antibodies first appeared a mean of 3.4 years prior to the diagnosis of SLE. These antibodies appeared early in the developmental course of disease compared to antidouble-stranded DNA antibodies, which were first detected a mean of 2.2 years before diagnosis. Anti-Sm and antinuclear ribonucleoprotein antibodies appeared a mean of 1.2 years prior to diagnosis, making them the latest predictors of

clinical disease. Also described was an accrual of autoantibodies throughout the years, which precedes clinical symptoms and halts upon diagnosis.[21]

Once SLE has been diagnosed, certain autoantibodies can be used as markers for disease activity or organ-specific clinical manifestation.[20] In a study by Ravirajan *et al.*,[22] there was a significant difference in heparan sulphate (HS) reactivity between patients with lupus nephritis compared with patients without renal disease. Also, patients with active disease and lupus nephritis had significantly higher levels of antinucleosome antibodies than patients with inactive disease and nephritis. Global disease activity score was seen to correlate with both antinucleosome and antihistone antibodies. The presence of renal disease correlated with antibodies to dsDNA and HS, but only the latter showed a significant correlation with disease activity in the kidney.[22]

In pregnant women with lupus, the presence of anti-Ro antibodies represents a significant risk factor for neonatal lupus and congenital heart block, prompting more careful monitoring. Monitoring antiphospholipid antibodies is of great importance in pregnancy on account of the greater risk of fetal loss, fetal growth retardation, and premature deliveries associated with the presence of these antibodies. In these cases, anticoagulant therapy may be advised.[20]

Furthermore, positive serology in pregnant women for anti-Ro and anti-La, has been found to be associated with future development of SLE and Sjögren's syndrome. Although most of the mothers with such serological findings are clinically healthy at the time of delivery, different studies have shown that the majority of these women will develop clinical SLE or Sjögren's syndrome in long-term follow-up.[10]

Central nervous system manifestations may be associated with certain antibodies and thus predicted. Among these antibodies are antiphospholipid antibodies associated with strokes, and antiribosomal P protein antibodies associated with cerebritis,[20] psychosis, and depression.[23]

Despite the predictive value of autoantibodies in SLE, and the correlation of specific antibodies with certain disease presentations (TABLE 1), no clinical intervention has been established as management in lack of overt manifestation. Nevertheless, the presence of autoantibodies in asymptomatic individuals as

TABLE 1. Autoantibodies as predictors of specific disease manifestations in SLE

Antibody	SLE manifestation
Anti-HS	lupus nephritis
Antinucleosome	lupus nephritis
Anti-Ro	neonatal lupus,* congenital heart block*
Anti-PL	pregnancy loss,* fetal growth retardation,* premature deliveries,* strokes
Antiribosomal P protein	cerebritis, psychosis, depression

HS = heparan sulphate; PL = phospholipids.
*Disease manifestations when antibody is present during pregnancy.

TABLE 2. Chronological pattern of appearance of autoantibodies predictive of SLE

Antibody	Mean years prior to clinical manifestation
Anti-PL	3.4 years
Anti-Ro	3.4 years
Anti-La	3.4 years
Anti-dsDNA	2.2 years
Anti-Sm	1.2 years
Antinuclear ribonucleoprotein	1.2 years

PL = phospholipids; dsDNA = double-stranded DNA.

well as the typical chronological pattern (TABLE 2) may justify the foundation of screening and follow-up programs for high-risk or general populations.

ANTIPHOSPHOLIPID SYNDROME

Antiphospholipid syndrome (APS) is a disorder of recurrent vascular thrombosis and pregnancy losses associated with persistently positive anticardiolipin or lupus anticoagulant tests.[24] A variety of abnormalities of the skin, the cardiac valves, central nervous system, and other organ systems have been also described.[25]

Many patients with APS have clinical and laboratory features found in other autoimmune disease, particularly SLE. Such patients are defined as having "secondary" APS, as apposed to patients with features of APS alone, thus defined as "primary" APS. Clinical and laboratory features in these two groups are similar.[24]

The similarity of these two entities has led to the notion that some primary APS patients may develop SLE or other autoimmune diseases, thus actually having secondary APS, which has yet to be exposed. A retrospective study[26] following 128 primary APS patients for a mean follow-up period of 9 years has investigated this notion. During the follow-up and after a median disease duration of 8.2 years (range, 1–14 years), 11 (8%) patients developed SLE, 6 (5%) developed lupus-like disease, and 1 (1%) developed myasthenia gravis. The remaining 110 patients (86%) continued to have primary APS. Of the risk factors related to the development of other autoimmune diseases, only the presence of Coomb's positivity had statistical significance (odds ratio, 66.4; 95% confidence interval [CI], 1.6–2714; $P = 0.027$) and was associated with the development of SLE. Hence, this study confirms that progression from primary APS to SLE or lupus-like disease is unusual, even after years of follow-up.[26]

Positive antiphospholipid tests have been known to be found in a variety of patients with drug-induced disorders and miscellaneous infectious disorders, such as syphilis, acquired immunodeficiency syndrome (AIDS), and others.[24] These patients, as opposed to APS patients, do not exhibit the clinical

phenomena of APS.[24] This special subgroup, along with healthy individuals positive for antiphospholipid antibodies, confounds the value of antiphospholipid antibodies in asymptomatic individuals as predictors of APS.

Antiphospholipid antibodies have been found to be associated with an increased risk of thrombotic phenomena, such as deep vein thrombosis, myocardial infarction, and stroke.[24] In a prospective nested case–control study by Ginsburg *et al.*,[27] the presence of anticardiolipin was found as a risk factor for deep vein thrombosis or pulmonary embolism in healthy adult men, regardless of age and smoking status. The risk for such a thrombotic event was directly correlated with antibody level, but a significantly increased risk was limited to values above the 95th percentile. In another 4-year-long prospective study,[28] the presence of elevated anticardiolipin antibody levels, 6 months following a venous thrombotic event, was found to be associated with an increased risk of recurrence and of death. The predictive values of the anticardiolipin antibody test increased with antibody levels.

In a large prospective study of SLE patients,[29] the presence of both lupus anticoagulant and anticardiolipin antibodies was found to be associated with an increased risk of venous thrombosis. However, of the two autoantibodies, lupus anticoagulant was found as a better predictor for venous thrombotic events.

In a prospective study performed by Brey *et al.*,[30] an association was demonstrated between the presence of IgG β2 glycoprotein-1-dependent anticardiolipin antibodies and the incidence of myocardial infarction (MI) and stroke. The risk factor for stroke after 15 years of follow-up in positive versus negative subjects for these antibodies was 2.2 (95% CI 1.5–3.4), and for MI was 1.8 (95% CI 1.2–2.6). The association between the presence of anticardiolipin antibodies and MI and stroke was shown to be attenuated in the last 5 years of follow-up.

In conclusion, although the presence of antiphospholipid antibodies may not be specific indicators of "primary" APS, their presence indicate greater risk of both venous and arterial thrombotic events. Therefore, testing for these autoantibodies may allow better risk stratification of healthy individuals in prevention programs, as well as better management of patients following thrombotic events in an attempt to prevent recurrence.

HEPATIC AUTOIMMUNE DISEASES

PBC is a chronic, progressive, cholestatic liver disease characterized histologically by fatal damage to the biliary epithelial cells lining the small intrahepatic bile ducts.[31] This damage is accompanied by a rich T cell mononuclear cell infiltrate with granuloma formation. Patients with late-stage disease can present with end-stage liver failure disease (portal hypertension and hepatocellular failure), although synthetic function is usually reserved till very late end-stages. Other characteristic symptoms are cholestatic itch and

profound fatigue, which bare no correlation to biochemical or histological disease severity.[31]

The principal autoantibody responses seen in PBC are directed against a specific mitochondrial (antimitochondrial antibodies [AMA]) and nuclear autoantigens (antinuclear antibodies [ANA]).[31] The main autoantigenic substrate for AMA is the E2 component of a mitochondrial enzyme complex—the pyruvate dehydrogenase complex (anti-PDC),[32] while ANA are directed against the nuclear pore membrane protein gp210. These autoantibodies, along with cholestatic blood biochemistry and appropriate histological findings comprise the diagnostic criteria for PBC.[31]

Numerous studies have attempted to prove the prognostic value of ANA and AMA, rather than their diagnostic value, yet so far most results are questionable. A possible exception is the association found between anti-gp210 antibodies and histologically advanced disease. Still, clinical significance is unclear.[31]

The predictive value of AMA was sought in a prospective study performed by Kisand *et al*.[32] A 9-year follow-up of asymptomatic, anti-PDC positive subjects was carried out. Three out of 8 subjects available for follow-up developed abnormal liver biochemical test results by the ninth follow-up year. Nevertheless, it is not clear whether anti-PDC antibodies mark the initiation of PBC, or rather reflect a predisposition to the disease.[32] Despite the ongoing prospective trials regarding the significance of different autoantibodies to disease development and prognosis, no clinical decisions are currently based upon serological findings.[31]

The sensitivity and specificity of AMA detected by indirect immunofluorescence and anti-PDC detected by ELISA for the diagnosis of PBC are both 95%. In comparison, the reported prevalence of ANA in PBC varies from 10–40%.[31] The predictive value of presence of AMA for PBC disease spectrum is demonstrated by the observation that 24 out of 29 patients who had AMA in the context of normal serum liver biochemistry were found to have histological features diagnostic of PBC on liver biopsy.[31] The vast majority of these patients went on over time to develop cholestatic liver biochemistry and classical symptoms of PBC.[31] The importance of such a finding lies in the ability to treat asymptomatic patients with drugs, such as ursodeoxycholic acid, and thus prevent or postpone the need for liver transplantation, and improve survival.[33]

The ability to identify asymptomatic individuals, the presence of a single specific test, and the ability to effectively treat asymptomatic patients leads to the notion of population screening. Since screening the general population is not cost-effective, specific screening of populations at-risk may be in order. For one, the 10:1 female to male ratio[34] suggests the screening of the female population alone. Furthermore, PBC shows strong heritability according to familial occurrence and monozygotic twin-concordance,[34] suggesting screening family members of diagnosed patients. Interestingly, and in contrast to other autoimmune diseases, PBC shows only weak associations with the usual

genetic risk elements for autoimmunity, such as the HLA alleles.[34] Hence, AMA screening of female individuals, especially family members of PBC patients may be greatly beneficial.

Autoimmune hepatitis (AIH) is an inflammatory liver disease characterized histologically by a dense mononuclear cell infiltrate in the portal tract and serologically by the presence of nonspecific autoantibodies and increased levels of transaminases and immunoglobulin G.[35] AIH is divided into two subgroups, AIH type 1 and AIH type 2, on the basis of the presence of ANA or antismooth muscle antibodies versus antibodies to liver/kidney microsome type 1 (LKM 1), respectively.

Primary sclerosing cholangitis (PSC) is a disease characterized by advanced fibroinflammatory damage of intra- and extrahepatic bile ducts, at times accompanied by autoimmune serology. Autoimmune sclerosing cholangitis (ASC) is a variant of PSC, typical of children and young adults, which is clinically characterized by less advanced bile duct lesions and laboratory findings similar to those of AIH type 1.[35]

Positivity to autoantibodies is critical for the diagnosis of AIH and ASC. Furthermore, the levels of these antibodies have been shown to mirror the extent of inflammatory activity in both diseases. The predictive role of autoantibodies in AIH and ASC is yet unknown, although antibody profiles suggestive of AIH type 1 have been found in asymptomatic family members of AIH type 1 patients. Furthermore, autoantibodies typical of AIH type 1 are often detected in formerly seronegative liver transplant patients, a proportion of whom will develop AIH. Still, more prospective studies are needed in order to fully establish the predictive role of autoantibodies in AIH of both types and ASC.[35]

INFLAMMATORY BOWEL DISEASES

Crohn's disease (CD) and ulcerative colitis (UC) are common clinical subtypes of idiopathic inflammatory bowel disease (IBD). These diseases are characterized by excessive, and tissue damaging inflammatory responses of the gastrointestinal tract.[36] Although the etiology is unknown, it is increasingly clear that these diseases represent the outcome of three essential interactive cofactors: environmental factors (e.g., enteric microflora), multigenic host susceptibility, and immune-mediated tissue injury.[36]

Several autoantibodies differentially associated with CD and UC have been investigated of which the most frequently studied in clinical trials are anti-*Saccharomyces cerevisiae* antibodies (ASCA) and perinuclear antineutrophil cytoplasmic antibodies (pANCA). These autoantibodies are considered as disease markers for IBD, although the clinical significance of their presence in healthy individuals up until recently has remained controversial.[36]

In a recent study by Israeli *et al.*,[36] sera of asymptomatic military personnel later diagnosed with CD or UC were obtained from a sera repository. The sera

were examined for ASCA and pANCA in an attempt to evaluate the predictive value of these autoantibodies for IBD. ASCA were found in the sera of CD patients up to 60 months prior to diagnosis, but were not found in any of the control sera. The estimated odds ratio calculated for CD in ASCA positive versus negative subjects was 30 (95% CI 4.27–1301.93). Also demonstrated was a significant rise in ASCA titer with time, and until clinical symptoms appear, suggesting that contrary to current beliefs ASCA are a marker of an autoimmune process occurring in IBD rather than a genetic marker alone.

Also demonstrated in this study was a significant association between pANCA and UC[36]; of 8 UC patients, 2 were pANCA positive prior to diagnosis, as opposed to none of the controls ($P = 0.014$).

In conclusion, the presence of autoantibodies, such as ASCA and pANCA in asymptomatic individuals may predict or rather precede clinical IBD. Such serological testing may allow early management or attentive follow-up programs, in an attempt to decrease morbidity associated with the disease as well as with surgical treatment.

AUTOIMMUNE ADDISON'S DISEASE

Primary adrenal insufficiency (PAI) is a clinical condition characterized by the inadequate secretion of corticosteroid hormones resulting from bilateral destruction or impaired function of the adrenal cortex. Among the many etiological agents recognized for this condition, a T cell-mediated autoimmune process (autoimmune Addison's disease [AAD]) is by far the most common cause in Western countries.[37]

AAD is associated with susceptible HLA haplotypes and is characterized by the appearance of autoantibodies to adrenal cortex cells (ACA).[38] ACA recognize an autoantigen located in the microsomal subcellular fraction of the adrenocortical cells, which has been identified as the steroid-synthesizing enzyme 21-hydroxylase (21OH). It has been shown that 21OH autoantibodies (21OHAb) have a high diagnostic sensitivity and specificity for autoimmune adrenal insufficiency. Most likely, the pathogenic role of adrenocortical autoantibodies is irrelevant, but their presence is a useful marker for disease classification at clinical onset.[38]

The determination of adrenal autoantibodies is critical to distinguish between subjects with ADD and subjects with nonautoimmune disease. The importance of a correct etiological classification is largely on account of the frequent association of ADD with other autoimmune endocrine diseases, in the so-called autoimmune polyglandular syndromes.[37]

Adrenal autoantibodies can be used to identify subjects with preclinical AAD, at high risk of progression toward clinical Addison's disease. It has been shown[38] that levels of adrenal autoantibodies correlate with the severity of adrenal dysfunction in the preclinical period. Also, early biochemical signs of

adrenal dysfunction have been shown to spontaneously remit, in parallel to the disappearance of both ACA and 21OHAb.

Because of the low prevalence of AAD in the general population and the low frequency of ACA or 21OHAb among healthy subjects, the predictive value of these markers has so far been studied only in patients with organ-specific autoimmune disorders.[37] It has been found that in children with hypoparathyroidism or type 1 diabetes mellitus, the predictive value of ACA for future clinical AAD is as high as 90% at 4 years, becoming 100% at 10 years. In adults, the predictive value of adrenal autoantibodies is lower than that observed in children and is around 20%.[37]

In a recent study of type 1 diabetes patients,[39] it was proposed that 21OHAb can be used as a marker for the large-scale screening of patients with endocrine autoimmune diseases for adrenal insufficiency. However, as also demonstrated by previous studies, the presence of adrenal autoantibodies does not lead necessarily to clinical Addison's disease.[39]

Recently reported[40] was the occurrence of long-term remission of subclinical adrenal failure with disappearance of 21OHAb and ACA in a patient with Graves' ophthalmopathy treated with corticosteroids. This effect of short-term glucocorticoid therapy can be attributed to the well-known immunosuppressive activity of steroids. Alternatively, corticosteroid therapy could act as an isohormonal therapy preventing progressive adrenal destruction with restoration to the normal state of adrenal function in subclinical autoimmune Addison's disease.[40] Such findings suggest the possibility of preventing clinical adrenal insufficiency. Nevertheless, such notions need to be independently confirmed.

Hence, adrenal autoantibody testing may serve as a useful tool to distinguish between subjects with ADD as apposed to a nonautoimmune disease, to identify subjects with preclinical AAD, and to predict future AAD in individuals with organ-specific autoimmune disorders, such as hypoparathyroidism or type 1 diabetes mellitus.

AUTOIMMUNE THYROID DISEASES

The term autoimmune thyroid diseases (AITD), encompasses a diverse range of clinical entities including among others Hashimoto's thyroiditis, juvenile thyroiditis, and Graves' disease. All AITD share a variable degree of lymphocytic infiltration of the thyroid gland along with thyroid antibody production. The presence of different AITD in members of the same family suggests a common etiological factor. The overlap extends to the occurrence of thyroid autoantibodies that are directed against three major autoantigens: thyroglobulin (TG), thyroid peroxidase (TPO), and the TSH-receptor (TSH-R).[41]

Several large studies have confirmed the value of anti-TG and anti-TPO antibodies in prediction of autoimmune hypothyroidism.[41] In a 20-year follow-up study in Whickman, UK, it has been shown that the odds ratio of

developing hypothyroidism in individuals with positive thyroid antibodies and normal TSH were 8 for women and 25 for men. These values rose up to 38 and 173, respectively, in the presence of elevated TSH levels and normal free T4 (subclinical hypothyroidism). These findings suggest the value of TSH surveillance in subjects with positive thyroid autoantibody serology.[41]

Anti-TPO antibodies found in pregnant women have been found to be correlated with postpartum AITD. It has been shown that 50% of pregnant women found positive for anti-TPO antibodies will develop postpartum thyroiditis.[41] The value of anti-TPO antibodies as predictors of thyroiditis depends upon their titer; a titer of 1:1600 or higher at delivery was found to have a 97% sensitivity and 91% specificity for postpartum AITD.[10] This close relationship between thyroid antibodies and postpartum thyroiditis has led to suggestions that all pregnant women should be offered antenatal screening for TPO autoantibodies, but as yet there is no consensus on its benefits.[41]

In conclusion, the presence of thyroid antibodies may be considered a risk factor for the development of future AITD, and thus renders a careful TSH follow-up. However, the benefits of screening for such antibodies in the general population or pregnant women have not been established.

PEMPHIGUS

Pemphigus comprises a group of chronic cutaneous bullous diseases that are characterized by autoantibody-induced epidermal cell to cell detachment (acantholysis). Pemphigus manifests itself clinically with flaccid blisters and erosions of the skin, histologically with acantholysis, and immunologically with bound and circulating IgG autoantibodies against various keratinocyte desmosomal antigens.[42]

The four major forms of pemphigus are pemphigus vulgaris (PV), pemphigus foliaceus (PF), and its endemic form fogoselvagem (FS), drug-induced pemphigus, and paraneoplastic pemphigus. The diagnosis of any of the clinical forms of pemphigus relies on clinical, histological, and immunological findings. Two desmosomal autoantigens have been characterized as the major targets of PV and PF autoantibodies: desmoglein 3 and desmoglein 1, respectively.[42]

The role of these antibodies as predictive markers has been explored in a study of a special population of Amerindians in Brazil in which the prevalence of FS is especially high. It has been found that antidesmoglein1 can be detected in patients months or years before onset of clinical disease. Also, clinical disease has been shown to be associated with a dramatic increase in these antibodies. In the same study, autoantibodies directed against desmoglein 1 were demonstrated in normal individuals, especially relatives of patients. These findings, along with the findings of a consecutive epitopal study have

demonstrated two different types of antidesmoglein 1 antibodies, only one of which is pathogenic. Thus, it has been suggested that individuals developing diseases are those with the appropriate genetic background. These individuals are exposed to environmental factors that allow the formation of pathogenic antibodies by means of epitope spreading.[42]

Hence, the presence of specific autoantibodies may predict pemphigus in asymptomatic individuals. Furthermore, the research of such antibodies in first-degree relatives of pemphigus patients may shed light on the pathogenesis of the disease and the role of genetic versus environmental factors in its development.

RECURRENT MISCARRIAGES

Many autoantibodies have been associated with impaired fertility. Among these are antiphospholipid antibodies, such as anticardiolipin,[43–45] antiphosphatidyl-serine,[46] antiprothrombin,[47,48] antilaminin-1,[49] and anti-β-2-glycoprotein 1, antibodies to thyroid antigens, such as anti-TG and anti-TPO, antibodies to extractable nuclear antigens (anti-ENA),[3,50] autoantibodies associated with SLE, such as anti-DNA,[51] and many more.[52]

Recurrent pregnancy losses are one of the most consistent complications of APS.[24] Losses can occur at any stage of pregnancy, although miscarriages associated with APS are strikingly frequent during the second and third trimester. However, the significance of positive serology for antiphospholipid antibodies on the outcome of the pregnancy depends greatly on previous obstetric outcome. It has been estimated that findings of a positive lupus anticoagulant test, or a moderate level of IgG anticardiolipin in a lupus patient are associated with a 30% risk of miscarriage during the first pregnancy. A history of two previous miscarriages in such a patient raises the risk of miscarriage during the following pregnancy to 70%. Although the risk of fetal loss is directly related to antibody titer, particularly the IgG anticardiolipin, many women with recurrent miscarriages have IgM anticardiolipin while others with persistently elevated antiphospholipid antibodies have no fetal complications at all. In conclusion, the best predictor of pregnancy outcome remains the previous obstetric history.

Several studies have found an association between spontaneous abortions and autoantibodies to the thyroid gland, such as anti-TPO or anti-TG, although the direct role of thyroid autoantibodies in fetal loss is debatable.[50] In a study by Tartakover-Matalon *et al.*,[50] it has been shown that active immunization of mice with human TG results in the production of anti-TG autoantibodies and pregnancy failure manifested by an increased fetal resorption rate (equivalent to human missed abortions) and reduced placental and embryo weights. The mice presented normal thyroid function and normal thyroid histology. This suggests that the higher rate of pregnancy loss observed in this model, as well as that described in women with thyroid antibodies, reflects primarily

an autoimmune phenomenon, rather than, or in addition to, a consequence of overt thyroid hormone abnormalities.[50]

Antilaminin antibodies, which may be detected in SLE and APS patients, have been previously shown to be associated with reproductive failures in animal models.[49] In a more recent study, a possible association has been made between anti-DNA antibodies in SLE and recurrent pregnancy loss.[51] It had been shown that exposure of human placentas to anti-DNA, an autoantibody previously shown to cross-react with laminin-1, resulted in abolishment of trophoblast attachment and migration. Laminins are basement membrane glycoproteins believed to play an important role in the remodeling of endometrial stroma, an important aspect of the implantation of the fertilized ovum into the uterus wall. Thus, anti-DNA antibodies which cross-react with laminin-1 may cause pregnancy loss in SLE patients by inhibiting this process.[51] Such knowledge may allow the prediction of possible pregnancy loss in women with high titers of anti-DNA or antilaminin antibodies, as well as contribute to future research regarding its prevention.

In a study testing different panel-kits in women suffering from impaired fertility, a significant association has been shown between recurrent miscarriages and autoantibodies to a combinational panel of anti-TPO, anti-TG, and anti-ENA.[3] In the same study antiphospholipid antibodies have been found to be associated with infertility.

The use of precise kits to anti-TG, anti-TPO, and anti-ENA autoantibodies as screening may diagnose immunologically mediated miscarriages,[3] and thus allow more appropriate and earlier management of such cases. Still, it is not clear which antibodies should be assessed in the evaluation and management of infertility and recurrent miscarriages.[3]

SUMMARY AND CONCLUSIONS

The Profile of an Individual Prone to Develop Autoimmune Diseases

It is now clear that autoimmune diseases, as does the end-organ pathologies they cause, do not begin at the time of clinical appearance, but rather many years before that. The implication of this concept lies in the possibility of predicting autoimmunity. Throughout the years, many risk factors have been found to be associated with autoimmune diseases. Of these, well documented were a female gender, a family history of autoimmune diseases and specific major histocompatibility complex (HLA) alleles.[7] Among these alleles are A1 and DQW1 associated with SLE,[7,53] DQ3-DR4, DQ3-DR9, DQ5-DR1, and DQ5-DR10 associated with RA,[54] DR3 and B8 associated with RA, SLE, autoimmune thyroiditis, celiac, multiple sclerosis, and myasthenia gravis, etc.[7] Other genetic risk factors researched are polymorphisms in specific genes encoding molecules involved in antigen presentation, such as Tap-1 and proteosomes, as well as many other candidate genes.[55–61]

Also documented were specific immune deficiencies associated with autoimmune diseases.[62] These immune deficiencies are genetically determined and in most cases precede or possibly lead to autoimmune phenomena. Complement system abnormalities, for example, have been linked to the development of SLE.[63] Among the different abnormalities, the most prevalent and most severe disease has been found to be associated with deficiency of the proteins of the C1 complex and with total C4 deficiency.[63] Antibodies directed against complement proteins have also been found in SLE patients.[63] Of these antibodies, the most important autoantibody to a complement protein is anti-C1q, which is found in approximately a third of patients with SLE.[63]

Other immune deficiencies associated with autoimmunity are common variable immune deficiencies associated with thrombocytopenia and hemolytic anemia, and selective IgA deficiency associated with SLE and RA.[62] Furthermore, individuals with selective IgA deficiency have been found to be positive for many autoantibodies despite lack of clinical diseases.[64,65] These findings may suggest a common immune disturbance, and/or a tendency to develop future overt autoimmune diseases. Furthermore, immune deficiency creates susceptibility to infections by various agents, thus increasing the risk of a secondary antiself immune response.[66]

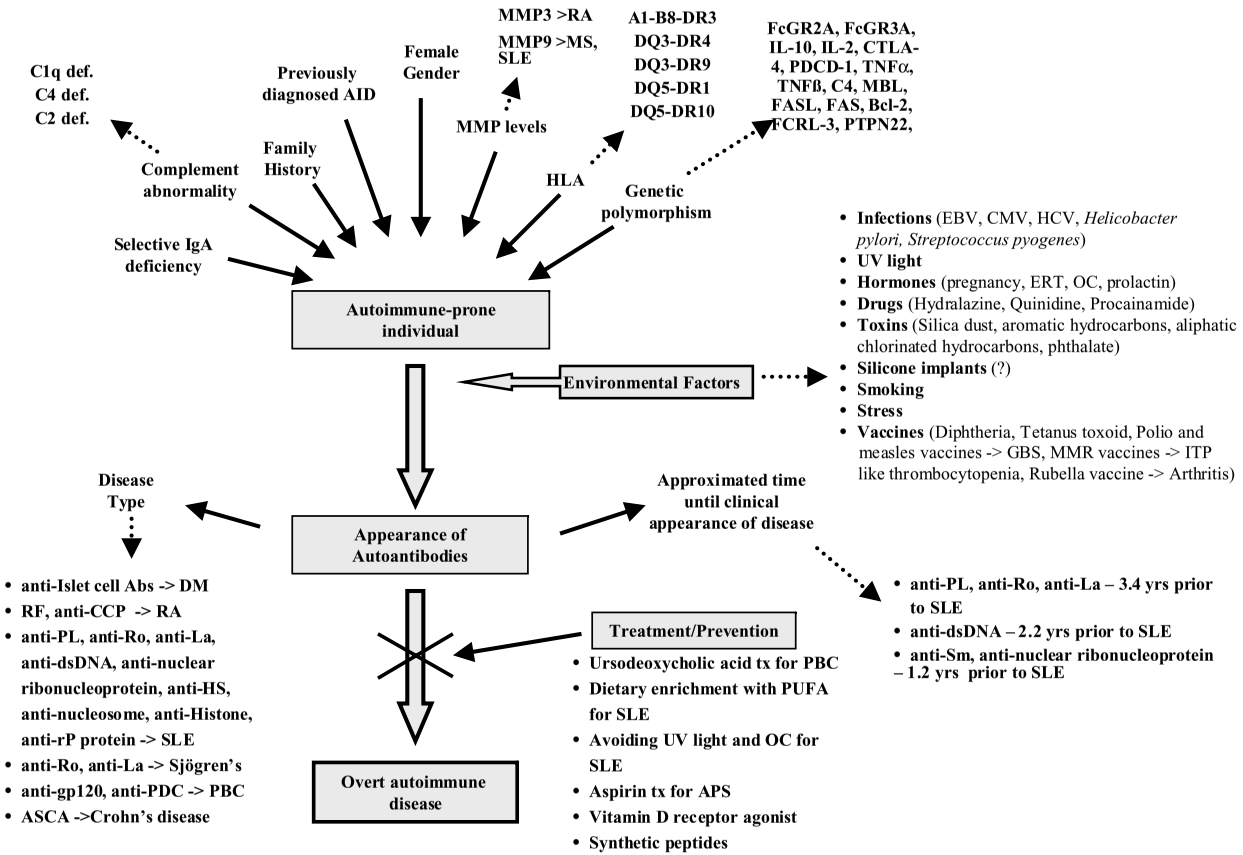
All of these risk factors and many others therefore create a profile of an "autoimmune-prone individual" (FIG. 1), which may be prone to develop an autoimmune disease upon exposure to a trigger antigen *via* infection,[66] vaccination,[67] or exposure to chemical substances.[67] While the specific autoimmune disease such an individual may develop depends upon the specific trigger antigen, as well as on the individual's genetics, the date of the disease's future clinical appearance and its specific manifestations may be predicted by specific serological tests (FIG. 1).

Indeed, it is now evident that many autoimmune diseases are preceded by a preclinical phase, which may be manifested by the presence of different autoantibodies (see TABLE 3). Nevertheless, these findings give rise to many questions regarding the management of individuals with positive autoantibody serology, as well as questions regarding future screening policies.

Screening for Autoantibodies as Predictors of Autoimmune Diseases—Practical and Ethical Issues

There is the real prospect that by screening the general population, identification of high-risk individuals for some diseases may be allowed. The goal of such identification would be either prevention of disease, or limitation of clinical impact.[2] However, while having answered the question "why," a number of other questions must be answered for appropriate strategies to be devised:

Who should be screened? Should screening include the general population, or rather first-degree relatives of patients, genetically prone HLA groups, etc? These special groups may benefit more from antibody screening compared to


C1q def.
C4 def.
C2 def.
Complement abnormality
Previously diagnosed AID
Family History
Female Gender
MMP3 >RA
MMP9 >MS, SLE
MMP levels
A1-B8-DR3
DQ3-DR4
DQ3-DR9
DQ5-DR1
DQ5-DR10
HLA
FcGR2A, FcGR3A, IL-10, IL-2, CTLA-4, PDCD-1, TNFα, TNFß, C4, MBL, FASL, FAS, Bcl-2, FCRL-3, PTPN22,
Genetic polymorphism
Selective IgA deficiency
Autoimmune-prone individual
Environmental Factors
• Infections (EBV, CMV, HCV, Helicobacter pylori, Streptococcus pyogenes)
• UV light
• Hormones (pregnancy, ERT, OC, prolactin)
• Drugs (Hydralazine, Quinidine, Procainamide)
• Toxins (Silica dust, aromatic hydrocarbons, aliphatic chlorinated hydrocarbons, phthalate)
• Silicone implants (?)
• Smoking
• Stress
• Vaccines (Diphtheria, Tetanus toxoid, Polio and measles vaccines -> GBS, MMR vaccines -> ITP like thrombocytopenia, Rubella vaccine -> Arthritis)
Disease Type
Appearance of Autoantibodies
Approximated time until clinical appearance of disease
• anti-Islet cell Abs -> DM
• RF, anti-CCP -> RA
• anti-PL, anti-Ro, anti-La, anti-dsDNA, anti-nuclear ribonucleoprotein, anti-HS, anti-nucleosome, anti-Histone, anti-rP protein -> SLE
• anti-Ro, anti-La -> Sjögren's
• anti-gp120, anti-PDC -> PBC
• ASCA ->Crohn's disease
• anti-PL, anti-Ro, anti-La – 3.4 yrs prior to SLE
• anti-dsDNA – 2.2 yrs prior to SLE
• anti-Sm, anti-nuclear ribonucleoprotein – 1.2 yrs prior to SLE
Treatment/Prevention
• Ursodeoxycholic acid tx for PBC
• Dietary enrichment with PUFA for SLE
• Avoiding UV light and OC for SLE
• Aspirin tx for APS
• Vitamin D receptor agonist
• Synthetic peptides
Overt autoimmune disease

the general population. Accordingly, testing high-risk groups may change the positive predictive value of autoantibody serological tests.

When should individuals be screened? The best age for screening varies in different diseases; for example, while diabetes-associated antibodies appear by 5 years of age, thyroid antibodies uncommonly appear before 20 years of age.[2]

Which antibodies should be screened for? Different autoantibodies appearing in the same diseases have different predictive values as individual screening test, as well as in combination.

How should the screening be performed? Specificity and sensitivity of different laboratory assays must be considered.

Who should be informed? Once autoantibodies have been found, a risk of future disease is established. This information may have great implication regarding one's future, and thus its distribution should be handled with great care. Should family members be informed, especially taking into account their associated risk? Should employment authorities be notified? Should such information be available to all caring physicians? Should military authorities be informed? Should one be obligated to inform insurance companies?

These seminal practical and ethical questions have to be resolved before any wide screening policy will be implemented.

The Prevention of Overt an Autoimmune Disease in a Prone Individual

As complex as it may be, the identification of positive autoantibody serology in an asymptomatic individual might allow immunological treatment whereby

FIGURE 1. Predicting and preventing autoimmunity. Risk factors, such as a female gender, certain HLA haplotypes, such as A1 B8 DR 3,[70] matrix metalloproteases (MMPs) activity,[71–73] specific immune deficiencies, and the presence of already one autoimmune disease, all create a profile of an "autoimmune-prone" individual. Upon exposure to an environmental factor, such as infection, vaccination, or a chemical substance,[67] such an individual may produce autoantibodies detectable by serological tests. These autoantibodies may serve as predicting markers of a specific autoimmune disease, as well as mean of approximation of its date of clinical appearance. Identification of these autoantibodies may allow the treatment and possibly prevention of the overt autoimmune disease. AID, autoimmune disease; MMP, matrix metalloprotease; RA, rheumatoid arthritis; MS, multiple sclerosis; SLE, systemic lupus erythematosus; EBV, Epstein Barr virus; CMV, cytomegalovirus; UV, ultraviolet; ERT, estrogen replacement therapy; OC, oral contraceptives; GBS, Guillian-Barré syndrome; MMR, measles/mumps/rubella; ITP, immune thrombocytopenic purpura; DM, diabetes mellitus; RF, rheumatoid factor; CCP, cyclic citrullinated peptide; PL, phospholipids; dsDNA, double-stranded DNA; HS, heparan sulphate; rP, ribosomal P protein; PDC, pyruvate dehydrogenase complex; PBC, primary biliary cirrhosis; ASCA, anti-*Saccharomyces cerevisiae* antibodies; PUFA, polyunsaturated fatty acids; APS, antiphospholipid syndrome.

TABLE 3. Predictive autoantibodies in autoimmune diseases

Disease	Autoantibodies
Type 1 diabetes mellitus	Anti-GAD
	IA-2
	IAA
RA	RF (anti-IgG)
	Anti-CCP
SLE	Anti-PL
	Anti-Ro
	Anti-La
	Anti-dsDNA
	Anti-Sm
	Antinuclear ribonucleoprotein
	Anti-HS
	Antinucleosome
	Antihistone
	Antiribosomal P protein
Sjögren's disease	Anti-Ro
	Anti-La
APS	Anticardiolipin
	Lupus anticoagulant
PBC	Anti-gp120
	Anti-PDC
AIH	ANA
Crohn's disease	ASCA
UC	pANCA
Autoimmune Addison's disease	ACA (anti-21-hydroxylase)
AITD	Anti-TG
	Anti-TPO
Pemphigus	Antidesmoglein 1

GAD = glutamic acid decarboxylase; IA-2 = protein tyrosine phosphatase-like molecule; IAA = insulin autoantibodies; RF = rheumatoid factor; CCP = cyclic citrullinated peptide; PL = phospholipids; HS = heparan sulphate; PDC = pyruvate dehydrogenase complex; ANA = antinuclear antibodies; ASCA = anti-*Saccharomyces cerevisiae* antibodies; pANCA = perinuclear antineutrophil cytoplasmic antibodies; ACA = adrenal cortex cells antibodies; TG = thyroglobulin; TPO = thyroid peroxidase.

disease is prevented, as is already being studied in diabetes.[10] Additionally, lifestyle modification may be recommended in order to prevent clinical disease or disease flare-ups. Such modifications include ursodeoxycholic acid treatment for PBC,[33] dietary enrichment with polyunsaturated fatty acids,[6] avoiding ultraviolet light exposure[7] and avoiding oral contraceptive agents for SLE,[8] and aspirin treatment in APS.[4] Still in question is the benefit of avoiding vaccination,[67] mainly on account of the relative risk of developing an autoimmune disease versus the risk of serious infection.

Another treatment or rather preventive modality currently being studied is the use of vitamin D receptor (VDR) agonists.[68] Different experimental models have shown the ability of VDR agonists to prevent different

autoimmune diseases, such as SLE in $MRL^{lpr/lpr}$ mice, experimental allergic encephalomyelitis (EAE), collagen-induced arthritis, Lyme arthritis, IBD, and autoimmune diabetes in non-obese diabetic (NOD) mice.[68] Furthermore, $1,25(OH)_2D_3$ analogs are able not only to prevent but also to treat ongoing autoimmune diseases, as demonstrated by their ability to inhibit type 1 diabetes development in adult NOD mice, and to inhibit the recurrence of autoimmune disease after islet transplantation in the NOD mouse. These analogs have also been shown to ameliorate significantly the chronic-relapsing EAE induced in Biozzi mice by spinal cord homogenate.[68]

Recently investigated is the benefit of synthetic peptides in the treatment of autoimmune diseases, and specifically APS.[69] Further research of this modality and its therapeutic and safety profile may enable its use earlier in the clinical course—prior to overt disease.

All of these treatment and preventive modalities underline the great importance that lies in autoantibody testing of asymptomatic individuals. Alternatively, even if disease cannot be prevented, by identification of individuals at risk, perhaps life-threatening conditions, such as thyroid storm and Addisonian's crisis could be avoided.[10]

In conclusion, although many issues remain unresolved, identification of autoimmune diseases in their preclinical stage has become feasible by autoantibody testing. The implementation of this ability is the treatment and possibly the prevention of autoimmune diseases. Nevertheless, many prospective studies are needed in order to assess the predictive value of antibody testing, as well as the means to apply them to clinical management of healthy population and high-risk individuals.

ACKNOWLEDGMENT

This work was partly supported by The Foundation Federico, S.A., for research in Autoimmunity Diseases.

REFERENCES

1. Notkins, A.L., A. Lernmark & D. Leslie. 2004. Preface. Autoimmunity **37:** 251–252.
2. Hawa, M., H. Beyan & R.D.G. Leslie. 2004. Principles of autoantibodies as disease-specific markers. Autoimmunity **37:** 253–256.
3. Marai, I. *et al*. 2004. Autoantibody panel screening in recurrent miscarriages. Am. J. Reprod. Immunol. **51:** 235–240.
4. Tincani, A. *et al*. 2003. Treatment of pregnant patients with antiphospholipid syndrome. Lupus **12:** 524–529.
5. Merlino, L.A. *et al*. 2004. Vitamin D intake is inversely associated with rheumatoid arthritis: results from the Iowa Women's Health Study. Arthritis Rheum. **50:** 72–77.

6. REIFEN, R. *et al*. 1998. Dietary polyunsaturated fatty acids decrease anti-dsDNA and anti-cardiolipin antibodies production in idiotype induced mouse model of systemic lupus erythematosus. Lupus **7:** 192–197.
7. LORBER, M., M.E. GERSHWIN & Y. SHOENFELD. 1994. The coexistence of systemic lupus erythematosus with other autoimmune diseases: the kaleidoscope of autoimmunity. Semin. Arthritis Rheum. **24:** 105–113.
8. PETRI, M. & C. ROBINSON. 1997. Oral contraceptives and systemic lupus erythematosus. Arthritis Rheum. **40:** 797–803.
9. NOTKINS, A.L. & A. LERNMARK. 2001. Autoimmune type 1 diabetes: resolved and unresolved issues. J. Clin. Invest. **108:** 1247–1252.
10. SCOFIELD, R.H. 2004. Autoantibodies as predictors of disease. Lancet **363:** 1544–1546.
11. DIABETES MELLITUS PREVENTION TRIAL – TYPE 1 DIABETES STUDY GROUP. 2002. Effects of insulin in relatives of patients with type 1 diabetes mellitus. N. Engl. J. Med. **346:** 1685–1691.
12. GALE, E.A. *et al*. 2004. European Nicotinamide Diabetes Intervention Trial (ENDIT): a randomized controlled trial of intervention before the onset of type 1 diabetes. Lancet **363:** 925–931.
13. LERNMARK, A. 2004. Type 1 diabetes as a model for prediction and diagnosis. Autoimmunity **37:** 341–345.
14. KUPILA, A. *et al*. 2001. Feasibility of genetic and immunological prediction of type I diabetes in a population-based birth cohort. Diabetologia **44:** 290–297.
15. BINGLEY, P.J. & A.J.K. WILLIAMS. 2004. Validation of autoantibody assays in type 1 diabetes: workshop programme. Autoimmunity **37:** 257–260.
16. ZENDMAN, A.J.W., E.R. VOSSENAAR & W.J. VAN VENROOIJ. 2004. Autoantibodies to citrullinated (poly) peptides: a key diagnostic and prognostic marker for rheumatoid arthritis. Autoimmunity **37:** 295–299.
17. NIELEN, M.M.J. *et al*. 2004. Specific autoantibodies precede the symptoms of rheumatoid arthritis, a study of serial measurements in blood donors. Arthritis Rheum. **50:** 380–386.
18. RANTAPÄÄ-DAHLQVIST, S. *et al*. 2003. Antibodies against cyclic citrullinated peptide and IgA rheumatoid factor predict the development of rheumatoid arthritis. Arthritis Rheum. **48:** 2741–2749.
19. ORBACH, H. *et al*. 2002. Gilburd B, Brickman CM, Gerli R, Shoenfeld Y, anti-Cyclic citrullinated peptide antibodies as a diagnostic test for rheumatoid arthritis and predictor of an erosive disease. Isr. Med. Assoc. J. **4:** 892–893.
20. TRAN, T.T. & D.S. PISETSKY. 2004. Systemic lupus erythematosus and related diseases. Autoimmunity **37:** 301–304.
21. ARBUCKLE, M.R. *et al*. 2003. Development of autoantibodies before the clinical onset of systemic lupus erythematosus. N. Engl. J. Med. **349:** 1526–1533.
22. RAVIRAJAN, C.T. *et al*. 2001. An analysis of clinical disease activity and nephritis-associated serum autoantibody profiles in patients with systemic lupus erythematosus: a cross-sectional study. Rheumatology **40:** 1405–1412.
23. EBERT, T., J. CHAPMAN & Y. SHOENFELD. 2005. Anti-ribosomal P protein and its role in psychiatric manifestations of systemic lupus erythematosus: myth or reality. Lupus **14:** 571–575.
24. KHAMASHTA, M.A., M.L. BERTOLACCINI & G.R.V. HUGHES. 2004. Antiphospholipid (Hughes) syndrome. Autoimmunity **37:** 309–312.
25. SHOENFELD, Y. 2003. Systemic antiphospholipid syndrome. Lupus **12:** 497–498.

26. GOMEZ-PUERTA, J.A. *et al*. 2005. Long-term follow-up in 128 patients with primary antiphospholipid syndrome: do they develop lupus? Medicine (Baltimore) **84:** 225–230.
27. GINSBURG, K.S. *et al*. 1992. Anticardiolipin antibodies and the risk for ischemic stroke and venous thrombosis. Ann. Intern. Med. **117:** 997–1002.
28. SCHULMAN, S., E. SVENUNGSSON & S. GRANQVIST. 1996. Anticardiolipin antibodies predict early recurrence of thromboembolism and death among patients with venous thromboembolism following anticoagulant therapy. Duration of Anticoagulation Study Group. Am. J. Med. **104:** 332–338.
29. SOMERS, E., L.S. MAGDER & M. PETRI. 2002. Antiphospholipid antibodies and incidence of venous thrombosis in a cohort of patients with systemic lupus erythematosus. J. Rheumatol. **29:** 2531–2536.
30. BREY, R.L. *et al*. 2001. β2-Glycoprotein 1-dependent cardiolipin antibodies and risk of ischemic stroke and myocardial infarction, The Honolulu Heart Program. Stroke **32:** 1701–1706.
31. JONES, D.E.J. 2004. Primary biliary cirrhosis. Autoimmunity **37:** 325–328.
32. KISAND, K.E. *et al*. 2001. The follow-up of asymptomatic persons with antibodies to pyryvate dehydeogenase in adult population samples. J. Gastroenterol. **36:** 248–254.
33. NISHIO, A. *et al*. 2001. Primary biliary cirrhosis: from induction to destruction. Semin. Gastrointest. Dis. **12:** 89–102.
34. INVERNIZZI, P. *et al*. 2005. From bases to basis: linking genetics to causation in primary biliary cirrhosis. Clin. Gastroenterol. Hepatol. **3:** 401–410.
35. VERGANI, D. & MIELI-VERGANI. 2004. Autoimmune hepatitis and sclerosing cholangitis. Autoimmunity **37:** 329–332.
36. ISRAELI, E. *et al*. 2005. Anti-*Saccharomyces cerevisiae* and antineutrophil cytoplasmic antibodies as predictors of inflammatory bowel disease. Gut **54:** 1232–1236.
37. MARZOTTI, S. & A. FALORNI. 2004. Addison's disease. Autoimmunity **37:** 333–336.
38. LAURETI, S. *et al*. 1998. Levels of adrenocortical autoantibodies correlate with the degree of adrenal dysfunction in subjects with preclinical Addison's disease. J. Clin. Endocrinol. Metab. **83:** 3507–3511.
39. BREWER, K.W., V.S. PARZIALE & G.S. EISENBARTH. 1997. Screening patients with insulin-dependent diabetes mellitus for adrenal insufficiency. N. Engl. J. Med. **337:** 202.
40. DE BELLIS, A.A. *et al*. 2001. Time course of 21-hydroxylase antibodies and long-term remission of subclinical autoimmune adrenalitis after corticosteroid therapy: case report. J. Clin. Endocrinol. Metab. **86:** 675–678.
41. WEETMAN, A.P. 2004. Autoimmune thyroid disease. Autoimmunity **37:** 337–340.
42. PIAS, E.K. *et al*. 2004. Humoral autoimmunity in pemphigus. Autoimmunity **37:** 283–286.
43. BLANK, M. *et al*. 1991. Induction of anti-phospholipid syndrome in naive mice with mouse lupus monoclonal and human polyclonal anti cardiolipin antibodies. Proc. Natl. Acad. Sci. USA **88:** 3069–3073.
44. BAKIMER, R. *et al*. 1992. Induction of primary antiphospholipid syndrome in mice by immunization with a human monoclonal anti-cardiolipin antibody (H-3). J. Clin. Invest. **89:** 1558–1663.
45. SHURTZ-SWIRSKY, R. *et al*. 1993. In vitro effect of anticardiolipin autoantibodies upon total and pulsatile placental hCG secretion during early pregnancy. Am. J. Reprod. Immunol. **29:** 206–210.

46. TARTAKOVER-MATALON, S. *et al*. 2004. Antiphosphatidylserine antibodies affect rat yolk sacs in culture: a mechanism for fetal loss in antiphopholipid syndrome. Am. J. Reprod. Immunol. **51:** 144–151.
47. VON-LANDENBERG, P. *et al*. 2003. Antiprothrombin antibodies are associated with pregnancy loss in patients with the antiphospholipid syndrome. Am. J. Reprod. Immunol. **49:** 51–56.
48. HAJ-YAHJA, S. *et al*. 2003. Anti-prothrombin antibodies cause thrombosis in a novel qualitative *ex-vivo* animal model. Lupus **12:** 364–369.
49. TARTAKOVER-MATALON, S. *et al*. 2003. Immunization of naïve mice with mouse laminin-1 affected pregnancy outcome in a mouse model. Am. J. Reprod. Immunol. **50:** 159–165.
50. TARTAKOVER-MATALON, S. *et al*. 2003. The pathogenic role of anti-thyroglobulin antibody on pregnancy: evidence from an active immunization model in mice. Hum. Reprod. **18:** 1094–1099.
51. QURESHI, F. *et al*. 2000. Anti-DNA antibodies cross-reacting with laminin inhibit trophoblast attachment and migration: implications for recurrent pregnancy loss in SLE patients. Am. J. Reprod. Immunol. **44:** 136–142.
52. SHOENFELD, Y. & M. BLANK. 2004. Autoantibodies associated with reproductive failure. Lupus **13:** 643–648.
53. WORRALL, J.G. *et al*. 1990. SLE: a rheumatological view. Analysis of the clinical features, serology and immunogenetics of 100 SLE patients during long-term follow-up. Q. J. Med. **74:** 319–330.
54. ZANELLI, E., F.C. BREEDVELD & R.R.P. DE VRIES. 2000. HLA association with autoimmune disease: a failure to protect? Rheumatology **39:** 1060–1066.
55. POWIS, S. J. *et al.*1992. Effect of polymorphism of an MHC-linked transporter on the peptides assembled in a class I molecule. Nature **357:** 211–215.
56. PEARCE, R.B. *et al*. 1993. Polymorphism in the mouse Tap-1 gene. Association with abnormal CD8+ T cell development in the nonobese nondiabetic mouse. J. Immunol. **15:** 5338–5347.
57. MAKSYMOWYCH, W.P. *et al*. 1994. Polymorphism in an HLA linked proteasome gene influences phenotypic expression of disease in HLA-B27 positive individuals. J. Rheumatol. **21:** 665–669.
58. NATH, S.K., J. KILPATRICK & J.B. HARELY. 2004. Genetics of human systemic lupus erythematosus: the emerging picture. Curr. Opin. Immunol. **16:** 794–800.
59. GREGERSEN, P.K. 2005. Pathways to gene identification in rheumatoid arthritis: PTPN22 and beyond. Immunol. Rev. **204:** 74–86.
60. KOCHI, Y. *et al*. 2005. Functional variant in FCRL3, encoding Fc receptor-like 3, is associated with rheumatoid arthritis and several autoimmunities. Nat. Genet. **37:** 478–485.
61. WANDSTRAT, A. & E. WAKELAND. 2003. The genetics of complex autoimmune diseases: non-MHC susceptibility genes. Nat. Immunol. **2:** 802–809.
62. ETZIONI, A. 2003. Immune deficiency and autoimmunity. Autoimmun. Rev. **2:** 364–369.
63. WALPORT, M.J. 2002. Complement and systemic lupus erythematosus. Arthritis Res. **4:** S279–S293.
64. GOSHEN, E. *et al*. 1989. Antinuclear and related autoantibodies in sera of healthy subjects with IgA deficiency. J. Autoimmun. **2:** 51–60.
65. BARKA, N. *et al*. 1995. Multireactive pattern of serum autoantibodies in asymptomatic individuals with immunoglobulin A deficiency. Clin. Diagn. Lab. Immunol. **2:** 469–472.

66. SHOENFELD, Y. & N.R. ROSE. 2004. Infection and Autoimmunity. Elsevier Publication. Amsterdam, The Netherlands.
67. MOLINA, V. & Y. SHOENFELD. 2005. Infection, vaccines and other environmental triggers of autoimmunity. Autoimmunity **38:** 235–245.
68. ADORINI, L. 2005. Intervention in autoimmunity: the potential of vitamin D receptor agonists. Cell Immunol. **233:** 115–124.
69. PIERANGELI, S.S. *et al*. 2004. A peptide that shares similarity with bacterial antigens reverses thrombogenic properties of antiphospholipid antibodies *in vivo*. J. Autoimmun. **22:** 217–225.
70. CANDORE, G. *et al*. 2002. Pathogenesis of autoimmune diseases associated with 8.1 ancestral haplotype: effect of multiple gene interactions. Autoimmun. Rev. **1:** 29–35.
71. GOETZL, E.J., M.J. BANDA & D. LEPPERT. 1996. Matrix metalloproteinases in immunity. J. Immunol. **156:** 1–4.
72. MATACHE, C. *et al*. 2003. Matrix metalloproteinase-9 and its natural inhibitor TIMP-1 expressed or secreted by peripheral blood mononuclear cells from patients with systemic lupus erythematosus. J. Autoimmun. **20:** 323–331.
73. DUBOIS, B., G. OPDENAKKER & H. CARTON. 1999. Gelatinase B in multiple sclerosis and experimental autoimmune encephalomyelitis. Acta. Neurol. Belg. **99:** 53–56.