Disease-associated autoantibodies become more and more important for routine diagnostics as well as basic and applied research. As many of these autoantibodies are detectable long time before clinical manifestations, they may be used to predict the development of the appropriate disease. However their potential role in the very early diagnosis or risk assessment of disease development remains to be further studied. The current knowledge, the facts and perspectives regarding the prediction of organ specific and systemic autoimmune diseases are discussed in Chapter 1. For disease prediction and early intervention it is necessary to understand the pathologic processes leading to autoimmune diseases. For instance, components of the innate immune system (e.g. Toll-like receptors) can have a dramatic impact on autoantibody response and disease pathogenesis, either by promoting or by regulating disease (Chapter 2). The different effects of autoantibodies in immune homeostasis and autoimmune manifestations are discussed in Chapters 3 to 6.1 (natural autoantibodies as catalytic or protective immunoglobulins, autoantibodies against protective molecules, macromolecular complexes, receptor structures and ion channels). Reviews and news regarding autoantibodies in organ specific (Chapter 6) and systemic autoimmune diseases (Chapter 7) follow. The main focus of Chapter 7 is the pathologic, diagnostic and prognostic relevance of autoantibodies against citrullinated proteins or peptides. Chapter 9 deals with methodical aspects and diagnostic stategies starting with general comments on early diagnosis of autoimmune rheumatic diseases. Technologies for the identification of novel autoantibodies as well as for the determination of autoantibodies and autoantibody profiles were presented. Improvement of autoantibody analyses by autoantigen designing and technological innovations were discussed. Optimized, standardized and cost-effective multiparametric assays are the prerequisite for a probable future use of autoantibodies for the more accurate prediction of diseases.

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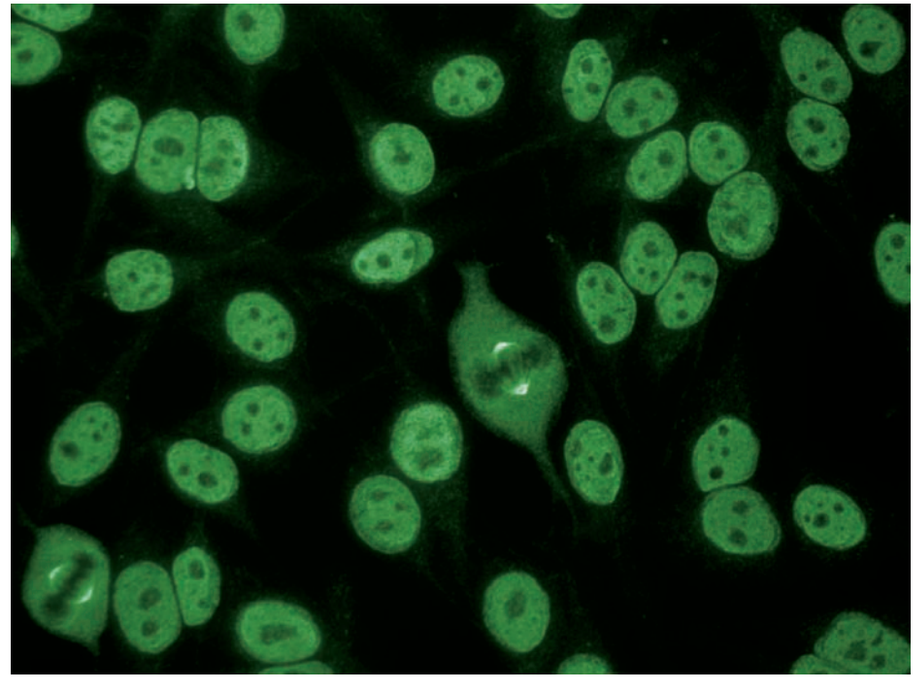

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Preface

For nearly five decades, autoantibodies with more or less specificity for disease or disease phenotype are used in routine diagnostics, basic as well as applied research. The applied research on autoantibodies has yielded an increasingly important approach to the diagnosis and management of patients with a variety of autoimmune conditions. The detection of autoantibodies in patient's sera is a key first step in most of the known autoimmune diseases. Although it has been shown that many disease-associated autoantibodies are detectable in preclinical stages, their potential role in the very early diagnosis or risk assessment of disease development is, with the exception of diabetes-associated autoantibodies, not entirely clear. Therefore, the fifth AAA volume has focused on the current knowledge about the predictive value of autoantibodies in organ specific and systemic autoimmune diseases. More research, especially long-term, multicenter, prospective studies, is necessary to evaluate the real value of autoantibodies for the prediction of disease development in very early stages.

Furthermore, all mechanisms and factors leading to the induction and maintenance of autoimmunity, as well as to the manifestation of autoimmune diseases, are important in order to develop novel options and strategies in prevention, early diagnostics, and effective therapy of these autoimmune diseases. In the last few recent years it has become clear that the innate immune system has a more important role in autoimmune processes as suggested in earlier reports. For instance, Toll-like receptors can have a dramatic impact on autoantibody response and disease pathogenesis, either by promoting or by regulating disease. Some components of the innate immune system can be regarded as a bridge between exogenous harmful factors and the development of specific autoimmunity. Autoantibodies, as biomarkers of these interactions, may be helpful in the elucidation of these complex pathogenic processes. Some of these aspects will be discussed in this volume along with examples of protective and pathogenic effects of autoantibodies.

In the future, autoantibodies may be used for a more accurate prediction of diseases and disease state, with the hope that early and effective intervention will be able to terminate ongoing pathologic processes. Autoantibody detection must be optimized, standardized, and ideally be cost effective, but even more important must be chosen from a clinical user perspective. Furthermore, the search

for novel autoantibodies with diagnostic or prognostic relevance must proceed. Emerging new technologies for the identification of novel autoantibodies, as well as for the determination of autoantibodies and autoantibody profiles, will be presented. Hopefully, the data and informations described and discussed here will stimulate novel concepts that will further the search for better prediction, prevention and treatment of autoimmune diseases.

The editors

Karsten Conrad
Edward K. L. Chan
Marvin J. Fritzler
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Chapter 1

Prediction of Autoimmune Diseases — Facts and Perspectives

Prediction of autoimmunity — more than just autoantibodies

Yehuda Shoenfeld

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A 53 years old mother with anti-phospholipid syndrome and vitiligo, came to my office for advise regarding her daughter, a beautiful 23 year old physical fitness trainer. A routine examination of the daughter revealed on ANA of 1:160, Lupus anticoagulant (LAC), IgA anti-CL and IgG anti-Tg. The mother asked me for my recommendations. Needless to say that the daughter was asymptomatic and sexually active.

Had I been asked for advice 10 years ago, my advice would be limited to a more follow-up, if anything (claiming that "We treat the patient and not the inflammation of the laboratory" — "Laboratitis"). However, the mother and the daughter sought my advice in 2007, after we had extensively reviewed the issue of prediction of autoimmune diseases [1–7]. Hence my advise entailed:
(1) Extension of the evaluation for autoantibodies to additional ones. (2) HLA analysis. (3) To avoid oral contraceptives. (4) If pregnant notify your physician (preferentially in a high risk pregnancy clinic) of being an asymptomatic auto-antibody carrier. (5) Avoid UV. (6) Avoid un-necessary vaccines [8–15]. (7) Keep a diet with enriched unsaturated fatty acid [16]. (8) Avoid smoking [17, 18]. (9) Continue with physical activities. (10) Add Vitamin D 400–800 IU a day to your diet [19].

Four months later, the young lady who was dating a nice fellow, had contracted from him infectious mononucleosis. Following this infection she developed thrombo-phlebitis and pulmonary embolus. Thus establishing a full blown clinical picture of anti-phospholipid syndrome (APS).

This clinical description exemplifies the importance of genetic background in autoimmunity (a mother with APS and vitiligo), the fortuitous detection of autoantibodies in an asymptomatic person, the impact of the triggering environmental factor (EBV infection) in the process of transforming the asymptomatic state of carrying autoantibodies to that of a well defined symptomatic (overt) autoimmune condition.

Previously, we have referred to the combined factors of the "mosaic of autoimmunity" [20, 21] as aiding in our ability to predict eventual evolvement of a mere presence of a specific autoantibody (or a combination of several autoantibodies) to an overt autoimmune condition. The "risk factors" entail:
(1) The many autoimmune genetic aspects from specific MHC marker to "notorious" haplotypes (i.e. HLA, A_1, B_8, DR_3), (2) TNFα, TNFβ, (3) C_4, C_1, C_2, (4) IgG receptors such as $F_c\gamma$RIIA, $F_c\gamma$RIIIA, (5) Manose binding lectin (MBL), (6) PTPN 22, (7) Osteopontin, (8) TYK_2, (9) IFR5, (10) CTLA-4, (11) CR_1, (12) Il-10, (13) FCRL3.

The hormonal factors include the sex hormones (leading to the higher prevalence of the disease in females) such as estrogens, but also prolactin [22–26]. Among the hormones one should also list the low levels of Vitamin D in all classical autoimmune conditions [19].

The immune system defects may encompass an immune deficiency state such as IgA deficiency, complement deficiencies (C1q, C4, C2), as well as aberrant reaction of the innate immunity (Toll like receptors).

Almost all classical autoantibodies were reported to precede the respective autoimmune disease by months to years [1–3]. Yet, the availability of the multiplex techniques [27, 28] enabling to measure several autoantibodies simultaneously, and the novel emerging protein and glycans cheap arrays — which will lead to the detection of hundreds of autoantibodies, the function of many of them still being obscure today — will lead to a decade of learning of new predicting autoantibodies.

Sophisticated algorithms and the employment of highly computerized formulas may lead by the end of the decade to a considerably improved ability of predicting the future development of autoimmune diseases, in more accurate manner. This ability to predict will be expanded not only to the type of the disease but most probably also to the tissues or organ(s) involved. The lag time of the disease development could be estimated more accurately, although exact time when environmental factors will intervene would remain relatively unknown, with the exception of the post-partum period.

The next decade may also expand our knowledge on the most important aspect of this issue: i.e. preventive measures. Currently our options are limited, avoidance of UV, stress, smoking, some vaccines, and oral contraceptives. We know from extensive experimental models and from limited diverse human studies, that Vitamin D may help to prevent autoimmunity. Yet we do not know the optimal doses (i.e. 400 IU or 2000 IU?). To determine those we will need to perform extensive epidemiological studies.

A more interesting question is whether one of the future biologics such as anti-Blyss, IVIG or one of the anti-cytokines will be valuable in reverting the course from autoimmunity to autoimmune disease.

The next decade will undoubtedly be exciting in all the above aspects, and will lead to better detection, prediction and hopefully better prevention of autoimmune diseases.

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Predictive autoantibodies: Past, present and future

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Key Words

predictive autoantibodies, thyroiditis, Graves' disease, type 1 diabetes mellitus, lupus, genetic markers, susceptibility genes, HLA

Introduction

Recent years have witnessed the introduction of many predictive biomarkers. They are defined as measurable characteristics that can assess normal function, pathologic changes or therapeutic responses predictively. Predictive markers are being applied to autoimmune diseases in a number of different areas including identification of susceptible individuals and populations, forecasting the outcome of clinical trials, aiding in developing new treatments and in the initiation of early or preventative interventions.

In the realm of autoimmune disease the most useful biomarkers to date have been autoantibodies. They are of special value because of their sensitivity and specificity. These terms are defined somewhat differently in a clinical or statistical context. Sensitivity is defined clinically as the proportion of subjects with a particular disease who have a positive test for that disease. In the instance of autoimmune disease, it is the number of subjects with a particular autoantibody

who have the disease divided by the total number of subjects who have the disease [1]. Specificity is the proportion of subjects without the disease who have a negative test result divided by the total number of subjects examined who do not have the disease. In many situations high sensitivity is accompanied by a high rate of so-called false positive reactions which represents a low specificity. Conversely high specificity may involve missing individuals at an early stage of disease or at a late timepoint when antibodies are low or simply missing persons who have poor antibody production. Applying these measures to predictive autoantibodies entails several problems. The first is that sensitivity and specificity may be defined by subjects who will develop the disease in the future rather than merely those who have the disease at the time of measurement. Obviously this figure is usually not known with any degree of certainty. Second, a single antibody may not be sufficient to define an autoimmune disease. Third, in rare diseases, even a small loss in specificity can make an antibody marker misleading because it will identify a relatively large number of individuals who do not have the disease in question, thereby giving a large number of false positives. Thus, the more compelling metric for predictive antibodies takes into account the prevalence of the disease [2]. The predictive value (PV) of positive result is given by the following formula:

$$PV = \frac{\text{true positives}}{\text{true positives} + \text{false positives}}$$

The predictive value of a negative result is:

$$PV = \frac{\text{true negatives}}{\text{true negatives} + \text{false negatives}}$$

Since most autoimmune diseases are rare, the positive predictive value; that is, the possibility of the disease when a particular test is positive, can be quite low. The usefulness of the positive predictive value is, therefore, dependent on the population selected. For example, if a test is applied to the general population, the prevalence of a particular autoimmune disease is generally low and, therefore, the positive predictive value also low. On the other hand, in a population made up of the family of patients, the prevalence can be many fold higher and the positive predictive value of an antibody consequently much greater. For that reason our earlier studies on predictive antibodies were carried out using the parents and siblings of children with autoimmune disease.

The past

Our first major studies on the application of predictive antibodies were carried out at Wayne State University in collaboration with my colleagues, Dr. Lynne Burek and Dr. William Hoffman [3–5]. Recruiting from a large pediatric endocrinology clinic, we were able to study 38 juvenile or adolescent patients with one of the

autoimmune thyroid diseases, chronic lymphocytic thyroiditis or Graves' disease, together with their siblings and parents. We soon found that family background was the strongest predictive marker in these cases of early onset thyroid disease. If both parents had primary evidence of thyroid autoimmunity in the form of thyroid autoantibodies (antibodies to thyroglobulin or to thyroperoxidase), there was a 75 % chance of one of the initially euthyroid siblings of our patient having thyroid antibodies. An euthyroid child in a family with only one parent with thyroid autoimmunity had a 54 % chance of developing autoantibodies; while in families in which neither parent showed evidence of thyroid autoantibodies, the initially normal child had only a 29 % chance of developing thyroid autoantibodies. This rate of antibody positivity was already much higher than found in children in which no autoimmune thyroid disease has been discovered in the family.

Since environmental factors are probably equally distributed among the three groups, we can attribute these differences in the prevalence of thyroid autoimmunity to heredity. In fact, two genetic markers of susceptibility had already been discovered at that time — the HLA haplotype and Gm allotype. We took advantage of our families to search for additional genetic traits that may raise the threshold of susceptibility to autoimmune thyroid disease or related autoimmune disorders. In those days before the availability of the complete human genome, we had to rely on individual genetic markers. In families of a child with autoimmune thyroid disease, we found associations with a number of well established genetic traits including ABO, Rh and Duffy blood groups (in addition to confirming the already known HLA and Gm associations). These studies as well as many others performed since have emphasized that most autoimmune diseases are related to the accretion of a number of genetic traits that raise (or sometimes decrease) the likelihood of occurrence of an autoimmune disease. An exciting recent finding is that a number of these genes, such as those determining CTLA4 and PTPN22, influence susceptibility to several autoimmune disorders [6]. The finding predicts that many of the traits adding to the"autoimmune diathesis" represent key genes regulating the immune response.

The role of HLA in the families of children with chronic lymphocytic thyroiditis was strikingly different from its role in the Graves' disease families. Susceptibility to Graves' thyrotoxicosis is clearly associated with HLA-B8 suggesting that this haplotype favors presentation of an immunodominant epitope on the thyrotropin receptor. In contrast, no single HLA haplotype predominated in the pediatric population susceptible to thyroiditis. However, 89 % of the siblings who shared both haplotypes with the proband had evidence of thyroid autoimmunity. Of the haplotype of the siblings who shared only one haplotype with the proband, 69 % had thyroid antibodies. In siblings sharing no haplotype with a patient, only 56 % had thyroid antibodies. Moreover, when they were examined in the clinic during later follow-up, 32 % of the siblings who had a two haplotype match with the proband had some biochemical or clinical evidence of thyroid dysfunction. If

only one haplotype was shared, one of ten siblings had subclinical thyroid disease, whereas in siblings sharing no haplotype with a patient, none had any evidence of thyroid dysfunction. Thus HLA is an important determinant of susceptibility to thyroiditis, but in the instance of thyroiditis the particular HLA haplotype conferring susceptibility differs from family to family. The findings suggest that different individual families respond to different antigenic determinants on the large thyroglobulin molecule.

Another important biomarker of susceptibility that emerged in our study was race or ethnicity. Of 18 patients with Graves' disease, 6 self identified as white and 12 as black. In contrast, among 20 thyroiditis patients, 18 were white and only 2 were black. Thus, among African American children with thyroid disease, most (75 %) were diagnosed with Graves' disease rather than thyroiditis, whereas among Caucasian children with an autoimmune thyroid disease, most (85 %) have thyroiditis.

Age of onset and sex are also predictive biomarkers. In prepubertal children the rate of males to females was nearly equal. After puberty two thirds of the patients with autoimmune thyroid disease were female.

Finally, thyroid autoantibodies themselves proved to be the most useful biomarker of susceptibility to present or future disease. Thyroid autoantibodies were found in all of the probands and in 50 % of the siblings who were euthyroid at the time of initial examination. Almost all cases of thyroiditis (75 %) or of Graves' disease (89 %) had antibodies to both thyroglobulin and thyroperoxidase; only a few individuals had antibodies to thyroglobulin or thyroperoxidase alone. In the siblings of a pediatric patient with autoimmune thyroid disease, the finding of both antibodies signified a greater risk of subclinical or impending disease than if no antibody or either antibody alone were detected.

In further studies, we were able to gain some information about the precise specificity of antibodies to thyroglobulin [7]. Using a large panel of monoclonal antibodies, Herbert Bressler in our team found that we could distinguish two general types of antigenic determinants on the thyroglobulin molecule. One group of determinants included thyroxine and was broadly cross-reactive among different mammalian species, whereas the other was fairly specific for human thyroglobulin. With Patrizio Caturegli [8] we were able to show that the antibodies present in many euthyroid individuals reacted with the evolutionarily conserved, broadly shared determinants on thyroglobulin, whereas sera from patients with thyroiditis or Graves' disease also recognized the clusters of epitopes that were predominantly human-specific. The shared determinants are probably the most primitive and relate to the core function of the thyroid-hormone whereas species-limited determinants are probably newer in an evolutionary sense.

Putting together the fruits of our own early investigation plus studies carried out since that time, one can assemble an instruction list of predictive biomarkers of autoimmune thyroid disease. They include: 1. family history; 2. age of onset

and sex; 3. presence of multiple thyroid-specific autoantibodies; 4. particular HLA haplotype; 5. non-HLA immunoregulatory genes; 6. ethnicity or race; and 7. production of autoantibodies to disease-associated epitopes.

The present

Our early studies on autoimmune thyroid disease serve as a model for investigating predictive biomarkers for a number of other autoimmune diseases. At the present time, the most advanced work relates to type 1 diabetes mellitus (TIDM) [9, 10]. The disease lends itself to this type of study because of its prolonged natural history and gradual progression. Individuals who are genetically susceptible to the disease develop autoantibodies relatively early, signifying the initiation of insulitis and beta cell injury. As the loss of beta cell mass continues, metabolic abnormalities begin due to a loss in insulin production and ultimately sufficient beta cell death produces overt hyperglycemia and diabetes.

Although most individuals with type 1 diabetes do not have a family history, the risk of disease in a first degree relative of a TIDM patient is approximately 15 % greater than the general population. The HLA haplotypes associated with the greatest risk are HLA-DR3 and HLA-DQ2, whereas HLA-DR2 is reported to be protective. In addition to the insulin gene, CTLA4 and PTPN22 have been associated with the risk of developing TIDM as well as autoimmune thyroid disease.

Autoantibodies represent the major predictive biomarkers of later TIDM in children. Studies carried out in the United States and Europe have shown that autoantibody production can begin as early as the first year of life and may foreshadow the development of diabetes as long as 15 years later. The progression to diabetes, moreover, is related to the number of autoantibodies produced. If antibodies are found to GAD, IA-2 and insulin, approximately 90 % of children will have overt diabetes after seven years of follow-up. If antibodies are produced to two of the antigens, approximately 75 % of children will be diabetic by the age of ten. If antibody is produced to only one of the three antigens, only about 25 % of the children will become diabetic during the 15 year follow-up.

The presence of TIDM is itself a risk factor for development of other endocrine autoimmune diseases. For example, autoimmune thyroid disease occurs in approximately 28 % of TIDM patients compared with approximately 5–6 % in the general population. Moreover, the development of thyroid disease in the diabetic population is predicted by the production of thyroid-specific autoantibodies, since individuals who produce antibodies to thyroperoxidase have about an 80 % probability of developing hypothyroidism compared with only about 10 % of the TPO negative population [11, 12].

Although Addison's disease is rare in the general population, it can be found in 14 to 21 % of patients with TIDM. The production of antibodies to the cytochrome

P450 enzyme 21 hydroxylase serves as an early biomarker for the development of adrenal insufficiency.

A second disease that has been well studied from the view point of predictive biomarkers is systemic lupus erythematosus (SLE). The genetic risk of SLE is conferred by HLA class II alleles DR2 or DR3. In addition, complement factor C4 deficiency increases the frequency of disease. Recent studies have shown that some lupus related autoantibodies precede the clinical manifestations of SLE by many years [13–15]. The autoantibodies with high predictive value include anti-Ro, anti-La, anti-nuclear antibodies, and antiphospholipid antibodies. Anti-double stranded DNA is intermediate in its predictive value, whereas anti-Sm and anti-nRNP are almost coincident with the first clinical evidence of lupus and typically appear within the year of clinical diagnosis. Interestingly the accumulation of autoantibodies seems to reach a plateau at about the time of diagnosis in most patients.

In the subset of patients who develop anti-Ro antibody, available data show that a particular octapeptide is consistently recognized. The peptide is closely related to a sequence found in Epstein-Barr virus nuclear antigen 1 and suggests that a cross reaction with this viral antigen may be an initiating factor in some cases of SLE [16, 17].

The list of other autoimmune diseases for which predictive autoantibodies have been investigated is growing rapidly [18–20]. They include, for example, rheumatoid arthritis (cyclic citrullinated peptide and rheumatoid factor), myositis, (tRNA synthetases), systemic sclerosis (topoisomerase), CREST syndrome (centromere proteins). In celiac disease studies have focused on tissue transglutaminase antibodies; in myasthenia gravis antibodies to the acetylcholine receptor. In pemphigus, desmoglein 3 has been studied as a prediction marker; in primary biliary cirrhosis E2 pyruvate dehydrogenase complex, and in vitiligo, tyrosinase. Yet, contrasting with earlier reports, in multiple sclerosis myelin basic protein and myelin oligodendritic glycoprotein antibodies are not always associated with progression to disease [21, 22]. In the relatives of patients with dilated cardiomyopathy, the presence of cardiac-specific antibodies has been reported to identify relatives at risk of progressing to heart failure [23]. In pregnancy antibodies to thyroperoxidase may be predictive of postpartum autoimmune thyroid disease and diabetes-associated antibodies predictive of gestational diabetes.

The future

Predictive autoantibodies will have multiple uses in future years. First, they may be valuable adjuncts in predicting the likelihood of developing clinical disease before the diagnostic signs are evident. They will have value in teaching us about the natural history of disease, particularly in providing information about the length of the prodromal period when the immune-mediated destructive process

is silently underway. They will also help in better classification of disease. For example, the presence of the characteristic diabetes antibodies in adult patients with diabetes may indicate late onset autoimmune diabetes. Predictive autoantibodies may provide useful information about the prognosis and future course of disease including the nature and severity of complications, and thereby help to design treatment. They may foretell the impending onset of a second or third autoimmune disease in patients with one autoimmune disorder. Finally, predictive autoantibodies may have value in selecting subjects for therapeutic trials in which the likelihood of disease is much greater than in the general population [24].

Another potential use of predictive antibodies is a more individualized approach to therapy using genetic, immunologic and biochemical markers to guide individual care.

In future years, broader application of predictive antibodies will be possible because of advances in proteomics and technologic improvement [25]. These newer developments allow testing a large number of autoantibodies ("multiplexing") in a relatively rapid and inexpensive manner. Individual profiles of antibody patterns may be predictive, improve diagnostic accuracy and serve as a guide to treatment. In addition, highly purified peptide subunits ot autoantigens can greatly increase sensitivity without a loss of specificity [26, 27].

These potential applications have lead to a renewed enthusiasm for the discovery and finer characterization of autoantibodies. There are, however, a number of cautions that must be carefully considered. With regard to prediction of future disease, this information is of value if appropriate interventions are possible [28]. In some cases, institution of early treatment can avoid many of the most devastating manifestations of the disease process. There is some indication that early treatment of rheumatoid arthritis can prevent the most crippling effects of the disease [29]. On the other hand, a number of clinical trials have been undertaken to determine if early treatment is useful in TIDM. So far these studies have not given clear-cut benefit [30]. To be acceptable, an early treatment must not only be able to arrest the autoimmune process and prevent the onset of clinical disease, but also be sufficiently safe and free of side effects to use in otherwise healthy individuals. As the use of predictive antibodies increases, the demands for devising safe and effective early interventions will rise.

An alternative approach to early treatment is to identify the environmental trigger factor of an autoimmune disease. If the patient can in some way be separated from the environmental agent responsible, the disease itself may never occur or not reoccur. For example, patients with celiac disease can be treated successfully by placing them on a gluten-free diet even though the inherited susceptibility to the disease is unaltered. Similarly, perennial treatment with antibiotics has prevented progression of rheumatic heart disease in many young people even though they retain their innate susceptibility to rheumatic fever.

Despite the concerns raised above research on predictive biomarkers will certainly accelerate in coming years, taking advantage of the accumulation of genetic, immunologic and biochemical knowledge. In their application to autoimmune disease they promise the greatest good of all — the possibility of preventing the irreversible destructive effects of the autoimmune process. In the past our approach to the treatment of autoimmune disease has too often been confined to remedying damage that has already occurred. The possibility that we can actively intervene to prevent irreversible damage is a goal worth striving for [31].

Summary

The predictive value of autoantibodies has been limited in the past by the relatively low prevalence of most of the autoimmune diseases. If studied within families, autoantibodies have been shown to be useful in predicting impending disease in thyroiditis, Graves' disease, type 1 diabetes mellitus and cardiomyopathy, among others. In a large populational study, autoantibodies have been demonstrated in lupus several years before the onset of clinical disease. The development of rapid through-put, multiple testing promises to open new opportunities for predictive use of autoantibodies. At the same time, the application of predictive approaches to disease raises serious policy and ethical concerns.

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