

Prediction and Prevention of Autoimmune Disease

A Personal Perspective

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ABSTRACT: Biomarkers, represented by genetically determined traits or biologic changes predictive of disease onset or outcome, are increasingly employed by academic and industrial investigators. They can identify unusually susceptible populations or individuals, facilitate prognosis, forecast the outcome of therapy in clinical trials, or aid in developing improved treatments or preventative measures. All of these applications have been applied to the autoimmune diseases. As a case in point, we have through the years used biomarkers to predict susceptibility and the longer-range outcome of thyroid autoimmunity employing, a number of different approaches. They have taught us valuable lessons for future broader applications of biomarkers. The clues for susceptibility include major histocompatibility complex (MHC) and non-MHC genes combined with the fine specificity of thyroid autoantibodies in siblings of patients with juvenile thyroid disease. Together these biomarkers are highly predictive of later thyroid autoimmunity and subclinical thyroid dysfunction. For example, the progression from benign autoimmunity to clinical thyroid disease is marked by the appearance of autoantibodies to species-restricted epitopes on thyroglobulin. Thus, predictive biomarkers aid in identifying individuals with inordinate risk of disease and provide opportunities for earlier interventions to arrest the disease process.

KEYWORDS: autoimmunity; thyroid; thyroiditis; Hashimoto's disease; hypothyroidism; Graves' disease; hyperthyroidism; thyroglobulin; thyroperoxidase

Portions of this article were published in a special supplement to the May 2007 issue of *The Scientist*.

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Ann. N.Y. Acad. Sci. 1109: 117–128 (2007). © 2007 New York Academy of Sciences.
doi: 10.1196/annals.1398.014

PROLOGUE

The dream began in the early 1960s when, as a medical student, I was assigned to help in the care of a 16-year-old girl. She had classical Hashimoto's thyroiditis, a disease that more commonly occurs among middle-aged women. I guessed that this early onset of disease signifies a particularly strong genetic predisposition. Further investigation indeed identified numerous cases of thyroid disease or other endocrinopathies in family members. It occurred to me that it would be possible to use genetic information to predict future development of autoimmune disease and, thereby, open opportunities to intervene before the pathologic process reached an irreversible level.

PREDICTION

The opportunity to pursue this idea arose 15 years later at Wayne State University School of Medicine. I joined with William H. Hoffman, a pediatric endocrinologist, who had an active clinic of children and adolescents with autoimmune thyroid disease and Lynne Burek, a skilled clinical laboratory immunologist. Later we added Avi Gimelfarb, a population geneticist. Looking at patients from Dr. Hoffman's clinic with either chronic lymphocytic thyroiditis or Graves' thyrotoxicosis, together with their siblings and parents, we found that a clinically euthyroid child in a family in which both parents had thyroid autoantibodies (as primary evidence of thyroid autoimmunity) had a 71% chance of having thyroid autoantibodies.^{1,2} A child from a family in which only one parent had thyroid autoimmunity had a 54% chance of developing autoantibodies, while in families in which neither parent showed evidence of thyroid autoantibodies, a child had a 29% chance of developing thyroid autoantibodies. Since we could assume that environmental factors operated equally among these three groups, we attributed these differences to genetics. Because thyroid autoimmunity was about the same in fathers as in mothers, we concluded that the genetic predisposition can be inherited from either parent, without regard to whether or not overt disease was present.

The basis for this prediction of an inherited predisposition to autoimmune disease had already been laid in the experimental laboratory. In 1971, Adrian Vladutiu and I were able to show that susceptibility or resistance to experimentally induced thyroiditis in mice was encoded in the H-2 gene segment.³ Further detailed studies showed that the major susceptibility gene co-localized with I-A so that haplotypes with k or s at this locus were generally good responders whereas strains bearing haplotypes with b or d at I-A responded poorly to the induction of thyroiditis by immunization with murine thyroglobulin.⁴ We later found that genes localizing with the I-E locus modify the induction of autoimmunity to thyroglobulin.^{5,6} Finally, genes at the H-2D and later H-2K locus were discovered to regulate the severity of thyroid lesions rather than the extent of the immune response itself.⁷ In subsequent years, of course, the distinction

between the role of class II major histocompatibility genes in presenting antigenic peptides to CD4 helper T cells and class I major histocompatibility complex (MHC) in presenting to CD8 cytotoxic T cells has been well described. At the same time, we showed that mouse strains matched at the MHC but differing in background genes varied significantly in susceptibility to thyroiditis. Clearly, this predilection to develop autoimmune disease is a multigenic trait with MHC playing a prominent role. In noninbred animals, like humans, we have suggested that the chance aggregation of a number of common genes probably operating through a variety of differing pathways regulates autoimmune responses.⁸

Soon after the studies of thyroiditis in the mouse were launched, we undertook parallel studies of the spontaneous form of thyroiditis present in noninbred OS chickens.⁹ Susceptibility to this spontaneous disease encoded at the B locus, a blood and tissue group in fowl.¹⁰ The B genotype was predictive of oncoming disease. However, the "family effect" indicated that additional genes unrelated to the MHC participated in dictating susceptibility to this disease, just as we predicted from the studies of human disease.¹¹

At the time there was already evidence of two genetic markers of susceptibility to Graves' disease, HLA haplotype, and Gm allotype.^{12,13} After documenting the role of HLA and Gm in our study group, we were able to look at a large number of additional genetic traits, located on different chromosomes, even in these prehuman genome days. The markers included six erythrocyte enzymes, five circulating serum proteins, and six blood groups. For families of a child with autoimmune thyroid disease, we found evidence of an association in the ABO, Rh, and Duffy blood groups, in addition to the known associations with HLA and serum allotypes.

The HLA associations in the families of children with chronic lymphocytic thyroiditis was strikingly different from Graves' disease.¹⁴ No single HLA haplotype predominated in the susceptible population. However, 89% of the siblings who shared both haplotypes with the proband had evidence of thyroid autoimmunity. Moreover, 32% of these siblings had biochemical or physical evidence of the thyroid dysfunction when they were subsequently examined in the clinic. Of the siblings who shared one haplotype with the proband, 69% had thyroid autoantibodies and only one had subclinical thyroid disease whereas in siblings sharing no haplotype with the patient, only 56% had thyroid autoantibodies and none had any evidence of thyroid dysfunction. Thus, HLA is an important determinant in thyroiditis, but the particular HLA haplotype conferring susceptibility differs from family to family. The findings suggest that different families may recognize different antigenic determinants on the thyroglobulin molecule.

Approximately 10 years after the original study Drs. Burek and Hoffman returned to the original population in order to determine the class II HLA haplotype and to follow up the clinical status of the siblings. Unfortunately, only a limited number of individuals were available for examination at that

time. Even in this small sample, it was evident that most siblings who share both HLA class II haplotype with the proband progressed from subclinical to clinical thyroid deficiency with an elevated TSH and depressed T4.

A number of other risk factors also emerged from these studies. Dr. Hoffman's clinic had roughly equal numbers of patients with European or African inheritance. Of 18 children with Graves' disease, 6 were self-identified as white and 12 as black. In contrast, among the 20 thyroiditis patients, 18 were white and only 2 were black. Thus, 86% of the African American children with autoimmune thyroid disease were diagnosed with Graves' disease rather than thyroiditis.

Age and sex are also important risk factors for thyroid autoimmunity using autoantibodies as an index. There was no difference in their frequency among prepubertal male and female siblings. There was, however, a significantly greater frequency of thyroid autoantibodies in the pubertal female siblings compared with the pubertal males.

Linked to a family history, the presence of autoantibodies is in itself the strongest risk factor for the development of thyroid disease. A number of autoantibodies were measured in the probands and family members. No associations of disease with antinuclear antibody, parietal cell antibody, smooth muscle, or mitochondrial antibody were found. However, antibodies to thyroglobulin and to thyroid peroxidase were significantly related to the presence of disease. An analysis of variance showed the relationship of both thyroid antibodies and the two autoimmune thyroid diseases, chronic lymphocytic (Hashimoto's) thyroiditis and Graves' disease. In order to analyze these associations more deeply, two-way contingency tables were constructed. Family members who had clinical or biochemical evidence of thyroid dysfunction were compared with nonaffected family members. Control families without evidence of thyroid disease were also included in the study. Thyroid antibodies were analyzed both for presence or absence and their estimated titer. Interestingly, there were no significant differences between families where the juvenile proband had chronic lymphocytic thyroiditis or thyrotoxicosis. However, there was a significant difference between affected and unaffected individuals in families with either disease, since the affected member had significantly greater frequency of autoantibodies. The unaffected members of the families in both groups of patients differed from the controls who were members of a non-affected family. Similarly, there was a significant association of disease with titer; high-titred thyroid autoantibodies were more common in affected family members than in the unaffected ones. This difference was seen in both chronic lymphocytic thyroiditis and thyrotoxicosis families. These results formed the first evidentiary basis for using thyroid autoantibodies as predictors of disease.

Thus, thyroid autoantibodies themselves emerged as the most important risk factors. Thyroid autoantibodies were found in all of the probands and in 50% of the siblings. These findings contrasted with the relatively infrequent presence (10%) of thyroid autoantibodies in control children and adolescents. In almost

all cases of thyroiditis (75%) or Graves' disease (89%) autoantibodies to both thyroglobulin and thyroperoxidase were demonstrated, but a few individuals had antibodies to thyroglobulin alone or thyroperoxidase alone. Among the siblings, the finding of both antibodies in the individual serum signified a greater risk of subclinical disease than if either antibody alone were detected. The presence of both thyroid-specific antibodies may be taken as an early warning sign of impending disease.

With the help of Dr. Ingrid Outschoorn, we determined the subclass of the thyroglobulin-specific antibodies. IgG1 was represented similarly in all patients with autoimmune thyroid disease. In patients with Graves' disease the predominant subclass was IgG4, whereas in Hashimoto's patients IgG2 was disproportionately represented.

In summary, a large body of experimental and clinical evidence available by the mid 1980s showed us that a number of genetic traits are predictive of later disease.¹⁵ Each gene contributes incrementally so that the aggregation of more genes leads to proportionately greater risk. Although HLA is an important risk factor, it is clearly not the only trait contributing to heightened susceptibility. Even today, only a few of the genes have been identified with certainty since linkage equilibrium and incomplete penetrance impedes studies on human genetics. Nevertheless, genetic traits are invaluable in predicting susceptibility. The presence of autoantibodies, in the absence of overt clinical disease, adds to the predictability. The presence of two thyroid-specific autoantibodies proved to be especially valuable as a marker of impending disease in individuals with an HLA susceptibility haplotype.

When we combined HLA haplotypes with antibody data as predictors of disease we could reliably identify siblings who were clinically normal at the time the study began, but who were discovered to have biochemical manifestations of thyroid dysfunction or a palpable goiter. Returning to these families a number of years later confirmed that many of them developed overt thyroid disease.

Drs. Burek and Hoffman put the concept of predictive antibodies to a further test by examining thyroid autoantibodies in children and adolescents with type 1 diabetes and their siblings.¹⁶ It had been recognized for many years that children with insulin-dependent diabetes have a high incidence of thyroid disease, suggesting a common autoimmune diathesis for the two endocrine diseases. They found striking differences between the prevalence of thyroid autoantibodies in families of European versus African extraction, pointing to a strong genetic component. Greater numbers of European-origin patients than African-origin patients had thyroid autoantibodies. Fourteen of 159 patients exhibited clinical hypothyroidism and all had antibody to both thyroglobulin and thyroperoxidase; they were predominantly white. The prevalence of thyroid autoantibodies was much greater among siblings of seropositive patients than seronegative patients, suggesting two subtypes of type 1 diabetes based on genetic predisposition.

The next step in our quest for methods of predictability came when Herbert Bresler joined our team.¹⁷ Using a large panel of monoclonal antibodies, he found that he could distinguish two general types of antigenic determinants on thyroglobulin. One group was broadly shared among different mammalian species and represented the evolutionarily conserved epitopes. A relatively smaller number of determinants were restricted to human thyroglobulin. Patrizio Caturegli added the essential information by studying serum samples from patients with Hashimoto's thyroiditis, Graves' disease, thyroid cancer, or healthy subjects.¹⁸ Briefly, all of the patients, as well as many of the normals, reacted with the conserved determinants that were broadly shared among thyroglobulins of different species. In addition to those reactions, patients with thyroiditis or Graves' disease recognized up to three clusters of epitopes that were human-specific. Interestingly, thyroglobulin autoantibodies in thyroid carcinoma patients recognized the same clusters as Hashimoto's patients, but patients with nontoxic goiter or healthy subjects showed no such reactions. Thus, the precise specificity of thyroglobulin antibodies may help to signify the development of clinically relevant thyroid disorders.

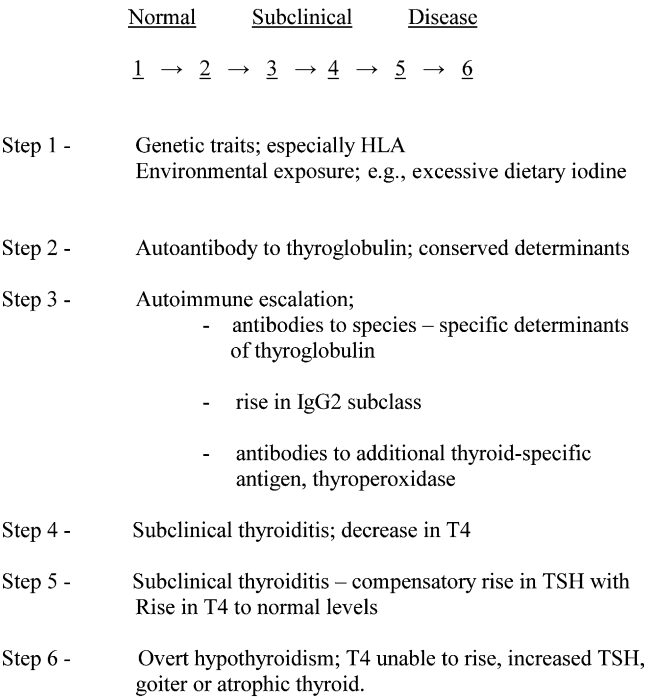
By combining findings from studies of patients with data from experimental animals, Dr. Burek and I constructed a model of the natural history of autoimmune thyroiditis (see FIG. 1) illustrating the opportunities for prediction of oncoming disease

PREVENTION

The endeavor to predict autoimmune disease before its clinical appearance should be linked to a program to prevent or abort the disease before irreversible destruction of the target organ has taken place. In humans, we have a few instances of interventions that successfully prevent progression of disease based on identifying the requisite environmental trigger. They include, as examples, prevention of rheumatic heart disease by perennial administration of antibiotics to prevent repeated streptococcal infections in children who have had rheumatic fever. Avoidance of gliadin by institution of gluten-free diets is successful in avoiding the clinical manifestations of celiac disease. Unfortunately, we do not have good examples of interventions that terminate an ongoing autoimmune response in humans. For example, efforts to treat diseases, such as rheumatoid arthritis, multiple sclerosis, and diabetes by oral administration of the putative antigen have not proved to be effective.

On the other hand, there is a great deal of experimental evidence suggesting that autoimmune responses can be prevented if antigen is given by a suitable route before disease is under way or even during its earliest stages. As an illustration, we have shown with Yi-chi Kong that intravenous injections of soluble murine thyroglobulin on day 7 and day 3 before initiating thyroiditis by immunization with murine thyroglobulin plus complete Freund adjuvant can completely prevent disease.¹⁹ The tolerance was antigen-specific and probably

Natural History of Autoimmune Thyroiditis



(Modified from original drawing by C. Lynne Burek)

FIGURE 1. Natural history of autoimmune thyroiditis.

dependent upon the presence of suppressor or regulatory T lymphocytes since the unresponsiveness could be transferred to syngeneic recipients with spleen cells.

More information on the origin and mechanism of action of these regulatory T cells was gleaned from studies carried out *in vitro* in collaboration with Eyal Talor.²⁰ The approach was based on our previous observations that neonatal thymectomy of OS chickens and BUF rats increases the severity of spontaneous thyroiditis suggesting that the thymus is a source of cells that can control the immune response. To identify these cells we used an enzyme-linked immunosorbent assay (ELISA) plaque essay for detecting antibodies specific for mouse thyroglobulin. Assuming that the immunoregulatory cells were present in the thymus, we immunized mice intravenously with an aqueous solution of mouse thyroglobulin in the absence of any adjuvant. Thymocytes from the injected mice effectively inhibited antibody secretion by B cells of immunized animals. The suppression was specific because the thymocytes did not inhibit antibody production to unrelated antigens, such as sheep red cells.

In time course experiments the maximum inhibitory activity was found in the thymus 9–16 days after injection of the soluble mouse thyroglobulin. Thymocytes taken at later times had distinctly less inhibitory activity. At the same time we found that the spleen cells acquired inhibitory activity as it was being lost from the thymus. Initially, inhibitory activity was found using spleen cells taken 14–16 days after immunization and was greatest 18–21 days after injection. We interpreted these findings as showing that thyroglobulin-specific regulatory T cells are generated first in the thymus and then migrate to the spleen. Further experiments showed that the inhibitory activity was associated with CD8⁺ T cells rather than CD4⁺ T cells and NK cells. Another striking finding from the study was the relative absence of these regulatory T cells from the thymus of SJL mice, which are known to be highly susceptible to a number of experimentally induced autoimmune diseases, such as thyroiditis.

Closer to the situation found in humans were experiments carried out in BUF rats. David Silverman and I found that when the young animals were injected with thyroid antigen, given in 7 or 8 weekly injections, disease was completely prevented.²¹ The effect was specific because other organ extracts had no effect. In contrast, if the thyroid antigen were administered after the onset of thyroiditis, the disease was unchanged or even intensified. In addition to intravenous administration, orally or nasally administered antigen may inhibit development of disease if given before disease was evident.

It is clear that we are only at the beginning of efforts to develop methods for preventing autoimmune disease, especially in highly susceptible individuals. Strategies may involve isolating environmental triggers or identifying the appropriate instigating antigen. Certainly preventing rather than treating autoimmune disease is a goal worth striving for.

EPILOGUE

The age of predictive antibodies is upon us.²² The basic approach developed three decades ago and described in the articles cited above has found application in a number of autoimmune diseases. The concept of prediction has reached its most advanced application in the assessment of the risk of type 1 diabetes. A recent article by Mrena, *et al.* illustrates the practical application of this approach.²³ The investigators have been performing a prospective study of childhood diabetes in Finland where the occurrence of disease is unusually high. The siblings of patients with type 1 diabetes were collected and stored for long periods of time. Disease-associated antibodies including the islet cell antibody assay (ICA), insulin-associated antibodies (IAA), antibodies to glutamic acid decarboxylase (GAD), and antibodies to protein tyrosine phosphatase (IA2) were performed and recorded. In this investigation, 47 siblings presented with clinical evidence of type 1 diabetes during a 15-year observation period. Of the 47 progressors, 38 tested initially positive for at least one

of the diabetes-associated autoantibodies and 7 initially autoantibody-negative siblings converted to positivity before diagnosis. Moreover, the combination of HLA haplotype and the number of disease-associated antibodies turned out to be highly significant predictors of the development to diabetes. In fact, the data suggested that the presence of two or more autoantibodies indicate a progressive, irreversible autoimmune process whereas positivity for only one of the diabetes-associated autoantibodies may reflect a harmless or even reversible autoimmune process.

There is a strong predicate supporting this line of investigation. Thus, a study in Germany by Jaeger *et al.* assesses the same for diabetes-associated antibodies in siblings of patients with type 1 diabetes.²⁴ Analysis showed a significantly increasing risk of diabetes in the siblings with the number of autoantibodies present. Moreover, individuals with more than one affected parent also had an increased risk of disease. Finally, an ongoing international study of diabetes' autoimmunity in the young Barker *et al.* has confirmed the increased risk of type 1 diabetes in individuals with a family history of the disease compared to the risk in the general population.²⁵ Most of the genetic risk could be attributed to the HLA region. The presence of diabetes-associated antibodies during infancy is highly predictive of the later development of the disease and the number and persistence of autoantibodies add to the risk. The DAISY study also confirmed that patients with type 1 diabetes frequently have other autoantibodies particularly to other endocrine organs, such as adrenal and thyroid. In addition, antibodies to the tissue transglutaminase antibody characteristic of celiac disease are also elevated.²⁵ As described above, there is reason to believe that the patients (perhaps as many as one-fourth) with antibodies to thyroid antigens represent a distinct clinical and genetic subset of type 1 diabetes patients. Thus antibodies may be useful not only in predicting imminent disease but in refining the diagnostic classification of a common syndrome. Patients with autoimmune thyroid disease often recognize distinct epitopes of GAD.²⁶

Another landmark in the developing saga of predictive antibodies was the publication by Arbuckle *et al.*²⁷ This team of investigators took advantage of a repository of serum samples collected by the United States Department of Defense. Tests were carried out for a large number of autoantibodies associated with systemic lupus erythematosus (SLE). In 115 of 130 patients with SLE at least one characteristic antibody was present before diagnosis. Sometimes the antibodies were demonstrable as long as 9 years before clinical appearance of disease. In the largest number of instances the earliest antibody was anti-Ro and anti-Sm. It turned out that the sequence recognized by anti-Sm is closely related to a sequence of the Epstein Barr virus nuclear antigen (EBNA-1).²⁸ These findings suggest that an immune response against the common Epstein Barr virus may in some individuals be an initiating factor of lupus autoimmunity.

The list of autoimmune diseases in which autoantibodies can serve as predictors has grown rapidly (refer to the special volume of Autoimmunity

37(4), 2004²⁹). For example, antibodies specific for cardiac antigens can be detected in many relatives of patients with dilated cardiomyopathy. Many of these relatives have echocardiographic changes suggestive of early cardiac disease.^{30,31} Similarly antibodies to β 2 glycoprotein 1 and phosphatidyl serine are predictors of antiphospholipid syndrome.³² Antibodies to cyclic citrullinated peptide seem to be valuable for the early diagnosis of rheumatoid arthritis.^{33,34} Antibodies to the F-actin, which is the major antigen detected by smooth muscle antibodies, show promise for the early diagnosis of autoimmune hepatitis.³⁵ Although the use of autoantibodies as predictors of autoimmune thyroid disease has not yet been moved into the realm of practical testing, they have shown value in prognosis. Autoantibodies to the TSH receptor may be valuable for prediction of relapse of Graves' disease in patients following the termination of antithyroid drug therapy.³⁶ Thus studies on the predictive value of autoantibodies begun so many years ago are now finding their way into clinical practice.

Simultaneously, information about the genetic precursors of autoimmune disease is rapidly adding to the fund of predictive risk factors. In addition to HLA, several polymorphisms contribute to the susceptibility to multiple autoimmune disorders. Polymorphism in the cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) gene on chromosome 2q33 as well as PTPN 22 encoding a lymphoid tyrosine phosphatase contributes to susceptibility to many different autoimmune diseases including type 1 diabetes, autoimmune thyroid disease, SLE, and rheumatoid arthritis.³⁷

Building on these successes must come renewed effort to evolve and evaluate procedures permitting early intervention. The methods must be sufficiently benign and yet sufficiently effective to arrest the progress of autoimmunity and thus to prevent autoimmune disease. Very likely such methods will arise from greater understanding of the many factors involved in the initiation of autoimmune disease. It will depend upon identifying the determinants that promote the autoimmune response before immune escalation and epitope spread make the possibility of early intervention impractical.

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