Linking Iodine with Autoimmune Thyroiditis

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A great deal of circumstantial evidence has linked iodine with the rising incidence of autoimmune thyroiditis in the United States. In our investigations, we have shown directly that T cells from humans with chronic lymphocytic thyroiditis proliferate in the presence of iodinated but not in the presence of noniodinated human thyroglobulin. Moreover, the proliferative response is restored when the thyroglobulin is iodinated artificially *in vitro*. Using a panel of monoclonal antibodies, we found evidence that the presence of iodine induces a number of stereochemical changes in the conformation of the molecule, resulting in the loss of some antigenic determinants and the appearance of others. One prominent determinant was associated with the iodine-containing amino acid thyroxine. Both the number and position of the iodine substituents determine the precise specificity of this epitope. A new model for the study of the role of iodine in inducing thyroid autoimmunity has become available in the form of the nonobese diabetic (NOD)-H2^{h4} mouse. This animal develops autoimmune thyroiditis spontaneously but in relatively low prevalence. However, if iodine is added to the drinking water, the prevalence and severity of the thyroid lesions increase markedly. The immune response is specific for thyroglobulin, both in terms of the antibody response and T-cell proliferation. In fact, the appearance of lesions can be predicted by the presence of thyroglobulin-specific IgG2b antibody. The disease, moreover, can be transferred adoptively, using spleen cells from iodine-fed donors treated *in vitro* with iodinated thyroglobulin. The effects of iodine feeding are greater in conventional animals compared with those maintained under specific pathogen-free conditions. Based on T-cell proliferation, it appears that the NOD-H2^{h4} strain of mice has innately a greater response to murine thyroglobulin than do other mouse strains and that the proliferation is increased even more by feeding iodine. We suggest, therefore, that the presence of iodine increases the autoantigenic potency of thyroglobulin, a major pathogenic antigen in the induction of autoimmune thyroiditis. This animal model provides a unique opportunity for investigating in detail the mechanisms by which an environmental agent can trigger a pathogenic autoimmune response in a susceptible host. *Key words*: autoimmune disease, autoimmunity, iodine, thyroglobulin, thyroiditis, thyronine, thyroxine, tyrosine. — Environ Health Perspect 107(suppl 5):749–752 (1999).

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Autoimmune Disease Is a Public Health Problem in the United States

The autoimmune diseases collectively represent a significant cause of chronic morbidity and disability in the United States. Unfortunately, few studies have measured quantitatively the impact of these diseases on the American population. Part of the problem is because autoimmunity as a major cause of human disease is a relatively recent discovery and very few agencies have concerned themselves with the autoimmune diseases as a group. Because autoimmune disease can affect virtually any organ of the body, its manifestations are protean. Autoimmune diseases therefore tend to be treated by physicians from many different specialties (*1*). Yet, because they are all linked by their etiology, it is important to consider them as a group when assessing the populational burden.

In a recent analysis, we estimated the number of persons affected by 24 well-established autoimmune diseases in the United States by applying mean weighted prevalence rates (*2*). The study was restricted to those diseases with well-defined evidence for an autoimmune pathogenicity and for which well-documented epidemiologic studies had been reported. Overall, we were able to estimate that approximately 8.5 million persons in the United States, or 1/31 Americans, are currently afflicted by one of the autoimmune disorders. The diseases with the highest prevalence rates are Graves disease (estimated prevalence 1,151.5/100,000); rheumatoid arthritis (estimated prevalence 860/100,000); and chronic lymphocytic thyroiditis (estimated prevalence 791.65/100,000). Moreover, it is evident that the occurrence of many of the autoimmune diseases is rising, although it is unclear whether it is due to greater recognition or an increased prevalence.

Genetic Predisposition and Environmental Triggers

One of the characteristics common to all autoimmune diseases is the clustering of several diseases with an autoimmune origin in the same individual or in family members. Although this clustering can be taken as prima facie evidence of a genetic predisposition, formal proof rests upon the demonstration of genetic determination of well-defined autoimmune diseases in animals. The first instance of clear genetic control was the demonstration that susceptibility to experimental thyroiditis in mice is associated with the H-2 haplotype (*3*). This study engendered many other investigations of animal models showing an enhanced susceptibility to several autoimmune diseases related to the class II major histocompatibility complex (MHC) determinants (*4*). Large-scale parallel investigations of human populations showed that many autoimmune diseases have marked predilection for certain human leukocyte antigen haplotypes (*5*).

In addition to genes of the MHC, experimental evidence shows clearly that non-MHC genes are involved in determining susceptibility to autoimmune diseases (*6*). Among the non-MHC genes that may be implicated are the immunoglobulin and T-cell receptor variable region genes, inherited differences in the ontogeny of the thymus, and genetic traits that influence the production of cytokines and related immunologic mediators.

The best method to assess the importance of genetic factors in determining susceptibility to autoimmune disease in humans is based on comparisons of monozygotic and dizygotic twins. In virtually every instance studied, the concurrence rate of monozygotic twins ranges from 15 to 50%, whereas dizygotic twins show little or no difference from other siblings (*6*). In broad terms, therefore, these data suggest that at least half of the susceptibility to autoimmune disease resides in nonheritable, epigenetic factors. These factors may include various forms of genetic reassortment, somatic mutation, or other stochastic events. It seems likely, however, that the greater proportion of the remaining susceptibility is due to environmental agents (*7*).

Among the environmental agents implicated in the induction of autoimmune disease, infectious organisms have historically been the most prominent (8). There is, for example, convincing epidemiologic evidence that pharyngitis due to the beta hemolytic streptococcus is a precipitating factor for rheumatic heart disease. It is widely assumed that this association is based on some form of molecular mimicry between the microorganism and the initiating autoantigen in the heart tissue of the host. However, at present, few, if any, clear-cut examples can be cited for this form of molecular mimicry causing human autoimmune disease (9).

Among the environmental agents known to act as environmental triggers of human autoimmune disease, the best established are drugs (10). Drug-induced autoimmune hemolytic anemia, thrombocytopenia, neutropenia, and systemic lupus erythematosus are well-established instances of external agents that initiate a pathogenic autoimmune response in susceptible hosts. When considering environmental agents, such as dietary components and pollutants, evidence for an association with autoimmune disease is more problematic. There is, moreover, very little understanding of biologic mechanisms by which environmental agents can serve as the triggers for autoimmune disease. Among foodstuffs, excessive iodine intake has been the best-studied example of a factor that increases the risk of autoimmune disease (11).

Iodine as an Environmental Trigger of Autoimmune Thyroiditis

The introduction of dietary iodine as a public health measure in the early twentieth century eliminated endemic goiter in the United States but may have spawned another set of problems (12). The incidence of autoimmune thyroiditis is increasing concomitantly with the progressively increasing iodine content in the American diet (13–15). Sources of dietary iodine include food and food additives (kelp and seaweed, iodinated salt, iodine additives to bread, flour, preservatives, and red coloring), therapeutics (amiodarone, vitamins, Lugol's iodine, etc.), topical antiseptics, and contrast dyes. In addition to the epidemiologic evidence, clinical studies have suggested a relationship of elevated iodine intake in autoimmune thyroid diseases (16–19). The effects of high iodine uptake, however, are observed only in genetically susceptible individuals (20). Conversely, in at least one clinical study, restriction of dietary iodine reversed hypothyroidism in 12 of 22 patients. When seven of the patients with reversed hypothyroidism were re-fed iodine, all of them became hypothyroid again (21).

Many mechanisms have been suggested to explain the association of iodine intake with autoimmune thyroiditis (22). They include damage by degeneration of free radicals, direct injury to thymocytes, and pharmacologic effects by inhibiting the sodium iodine pump. Studies in our own laboratories have strongly implicated a role of iodine in promoting thyroid autoimmunity by enhancing the autoimmunogenic properties of thyroglobulin (23,24).

Thyroglobulin, a major protein constituent of the thyroid gland, is a highly conserved protein among different mammalian species. It serves as a biochemical storage form of the circulating thyroid hormone thyroxine. It is a homodimer, each chain comprising 2,748 amino acids, of which 67 are tyrosines. Only four of the tyrosines per chain, however, are believed to play a role in the generation of thyroxine (25). These four tyrosines have high affinity for iodine. Early iodination evidently takes place at these specific sites and in a particular sequence (26,27). However, many other tyrosyl sites are available for storage of iodine. The affinity of these other tyrosyl residues varies considerably according to their accessibility, neighboring groups, and ionization constants. With increasing degrees of iodination, modifications in structure may occur that change the properties of thyroglobulin and lead to new molecular forms and to changes in stereochemical shape (28,29). Increased binding of iodine to tyrosyl residues enhances the stability of thyroglobulin and reduces its susceptibility to the proteolytic cathepsins of the thyroid (30). Increased iodination of thyroglobulin can thus heighten its autoimmunogenic potential by changes in antigen processing, alterations in stereochemical shape, production of novel iodine-containing determinants, or appearance of cryptic epitopes.

Iodine Is Required for Human T-Cell Recognition of Thyroglobulin

The important role of iodine in conferring antigenicity on thyroglobulin was first demonstrated by Roitt and Cooke (31). They found that murine thyroglobulin-reactive T cells proliferated with a human thyroglobulin, depending upon the degree of iodination of the molecule. In order to validate the studies in humans, we developed a thyroglobulin-specific proliferation assay, using human peripheral blood lymphocytes (32). T cells from four of five patients with chronic lymphocytic thyroiditis showed significant proliferation with normal human thyroglobulin. Three of five control samples from euthyroid individuals also provided evidence of proliferation but significantly less than found in the patients with thyroiditis. These findings suggest that

many normal humans have T cells reactive with human thyroglobulin. In contrast, none of the human T cells gave any measurable proliferation in response to preparations of thyroglobulin that contained no detectable iodine even after addition of interleukin-2 to increase the sensitivity of the assay procedure. The essential role of iodine was established when the iodine-free thyroglobulin was iodinated in vitro. It then produced a significant proliferative response with both patient and control lymphocytes, comparable in magnitude to that found with naturally iodinated thyroglobulin. Our results demonstrate that iodinated thyroglobulin is required for its recognition by human T cells.

Effects of Iodination on the Immunoreactivity of Human Thyroglobulin

We have considered two possibilities to account for the increased antigenicity of iodinated thyroglobulin. First, iodination may alter the stereochemical configuration of thyroglobulin, thereby affecting the manner by which it is processed and presented to T cells. The second possibility is that iodine creates a novel epitope by its presence in a particular antigenic determinant. These two possibilities were investigated using a panel of murine monoclonal antibodies to human thyroglobulin. These antibodies were carefully evaluated by competitive inhibition test to delineate the particular epitopes that they bound (33). Some of the monoclonal antibodies reacted with sites on thyroglobulin that were widely shared among thyroglobulins of different species and accounted for the extensive cross-reactivity of this molecule. They represent the most conserved portions of thyroglobulin and, in many cases, are associated with its physiologic function because they contain thyroxine. Other monoclonals reacted with sites that were relatively specific for the human antigen. These sites were the same ones recognized by antibodies from patients with autoimmune thyroid disease (34). These monoclonals were not inhibited competitively by thyroxine.

Detailed analysis demonstrated that iodinated thyroglobulin differs from non-iodinated thyroglobulin in its reactivity with this panel of monoclonals and indicated a loss of some epitopes and gain of others (35). Thus, the insertion of iodine induces significant stereochemical changes in the thyroglobulin molecule, resulting in changes in its immunoreactivity.

One of the monoclonals used in the study was particularly valuable because it reacted with iodinated thyroglobulin but failed to react with the noniodinated molecule. Moreover, its reaction with non-iodinated thyroglobulin was restored when

thyroglobulin was iodinated artificially *in vitro*. The fine specificity of this antibody was assessed using a series of iodinated thyronines and tyrosines (*36*). Briefly, it was found that the greatest affinity in terms of competitive inhibition was produced by 3',5',3,5-tetraiodothyronine or thyroxine. Significantly less binding was produced with 3,5',5-triiodothyronine, and even less by reverse triiodothyronine, 3,3',5'-triiodothyronine. Not only the number but the position of the iodine substitutions on thyronine determines its binding affinity. Diiodothyronine showed very little binding and none was measured with noniodinated thyronine. Thus, iodine is capable of producing a unique epitope recognized by this monoclonal antibody. We suggest that the human T cell may recognize a similar iodine-containing antigenic determinant.

The NOD-H2^{h4} Mouse: A New Model for the Study of the Role of Iodine in Autoimmune Thyroiditis

The nonobese diabetic (NOD) mouse is widely used as a model for type 1 diabetes mellitus in humans. The disease in these mice shares many features with human diabetes, including insulitis, islet cell antibodies, and genetic susceptibility conferred by both MHC and non-MHC genes. The NOD mouse expresses a unique class II MHC molecule, I-A^{g7}. Dr. Linda Wicker at Merck Laboratories created a series of congenics in which the class II MHC region was replaced by the MHC of other mouse strains [described in Weatherall et al. (*37*)]. One of these strains, designated NOD-H2^{h4}, was produced by crossing NOD with B10.A(4R), an H-2^k mouse. None of these mice developed diabetes. An unexpected finding was a high incidence of spontaneous thyroiditis in the NOD-H2^{h4} strain not found in either of the parental strains. Furthermore, the incidence of thyroiditis rose when iodine was added to the diet. If the genetic predisposition is present, therefore, an elevated intake of iodine can tip the scales toward greater T-cell stimulation and progression to thyroid disease.

To investigate the parameters of this unique model of a diet-induced autoimmune disease in a genetically susceptible strain, we added varying doses of iodine to the drinking water of NOD-H2^{h4} congenic mice 8 weeks of age and measured the prevalence of thyroiditis. The prevalence of disease increased with the dose and duration of feeding, leading to a maximum prevalence of > 90%.

The production of thyroid-specific antibodies was followed over time (*38*). The level of antibodies to thyroglobulin paralleled the increasing prevalence of disease. We were unable to detect antibodies to a second thyroid antigen, thyroperoxidase, and no direct correlation could be demonstrated between the lesion grade and the level of thyroglobulin-specific antibody. However, when we examined the individual isotypes of thyroglobulin-specific antibodies, we found a significant correlation between the presence of thyroglobulin-specific IgG2b antibody and the occurrence of thyroiditis.

These results strongly implicate the pathogenic role of thyroglobulin in this disease. In addition to the investigations of thyroglobulin-specific antibody, adoptive transfer experiments were conducted with spleen cells from iodine-fed donor NOD-H2^{h4} mice to younger recipients (*39*). These younger animals had a very low incidence of thyroiditis. When given spleen cells from iodine-fed donors, however, the incidence of disease increased significantly, illustrating that the disease can be transferred by adoptive immunity. The relative importance of cell-mediated and antibody-mediated immune responses has not yet been defined in this new mouse model.

To evaluate the reactivity of T cells to murine thyroglobulin, NOD-H2^{h4} mice were compared with three other strains. We found that spleen cells of NOD-H2^{h4} mice had an innate ability to react with murine and human thyroglobulin compared with the three other strains tested. Moreover, following treatment with iodine, the innate proliferation of the NOD-H2^{h4} to thyroglobulin increased to more than double that of the noniodinated treated animals.

In another series of experiments, we evaluated the effects of specific pathogen-free (SPF) and non-SPF conditions on autoantibody production and disease development. Iodine treatment under non-SPF conditions resulted in a high prevalence and greater severity of thyroiditis (*40*). The appearance of thyroglobulin-specific IgG2b was also frequent in the non-SPF iodine-treated group, corresponding with the greater incidence of thyroid lesions. This finding contrasts with the situation in conventional NOD animals, which develop diabetes primarily under SPF conditions.

In our opinion, this new mouse model will be of great value in elucidating the biologic mechanisms by which an environmental agent can induce autoimmune disease in a susceptible host.

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