

SHORT ANALYTICAL REVIEW

Anti-thyroglobulin Autoantibodies in Autoimmune Thyroid Diseases: Cross-Reactive or Pathogenic?

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1. INTRODUCTION

Hashimoto's thyroiditis (HT), the most common form of autoimmune thyroid disease (AITD), is characterized by infiltration of the thyroid by lymphocytes, gradual destruction of the gland, and production of various thyroid autoantibodies, notably anti-thyroid peroxidase (TPO) and anti-thyroglobulin (Tg) antibodies (1). It is currently not known whether these disease-defining autoantibodies are directly involved in the pathogenesis of the disease or are nonpathogenic antibodies generated secondary to the tissue damage caused by thyroid-infiltrating T lymphocytes. Likewise, the role of anti-Tg antibodies in the development of experimental autoimmune thyroiditis induced in mice by immunization with thyroglobulin is still debatable (2). This review will discuss the pertinent data on the binding properties of anti-Tg autoantibodies and on their possible pathogenic role in the development of AITD.

2. THE ANTIGEN

Tg, the main protein synthesized in the thyroid gland, serves both in the synthesis and in the storage of thyroid hormones (3). Tg is a macromolecular glycoprotein dimer of two identical 330-kDa polypeptide chains and is unique in its content of iodinated amino acids. Most iodinated amino acids in Tg are iodotyrosines, which serve as precursors of the biologically active hormones thyroxine (T4) and triiodothyronine (T3) (4). The gene coding for the Tg protein has been cloned and sequenced (5, 6). The human Tg gene is located on the long arm of chromosome 8 (7), and it is transcribed into an mRNA of approximately 8.5 kb which codes for a polypeptide of 2767 amino acids representing the Tg monomer of 330 kDa (8). The Tg monomer undergoes posttranslational modifications, including glycosylation, iodination, phosphorylation, and sulfation, and these may be important in its anti-

genicity (9–12). By far the most important posttranslational modification of Tg is iodination. The iodination of tyrosine residues is catalyzed by a membrane-bound enzyme, TPO, which has been shown to be the microsomal antigen (13, 14). However, not all of the iodinated tyrosine residues can be used for hormonogenesis. Recent studies have suggested that the human Tg molecule contains only four thyroid hormone synthesis sites from the iodinated tyrosines at positions 5, 2553, 2567, and 2746 (10, 15, 16). Nevertheless other tyrosine residues have also been suggested as possible hormonogenic sites in Tg molecules from different species (17, 18). It appears that the hormone synthesis sites and the iodine content of Tg play an important role in its antigenicity (19–21).

3. THE ANTIBODIES

Human Tg is one of the main autoantigens in AITD (22), and serologic studies have shown that there are at least 40 antigenic epitopes on human Tg (23). Three approaches have been used to study antigenic domains on the thyroglobulin molecule: (1) analysis of the binding of Tg-reactive sera (from patients with AITD or from normal individuals) with fragments of the Tg molecule, (2) cross-inhibition studies using panels of anti-Tg monoclonal antibodies to identify conformational epitopes or reactivity clusters on the Tg molecule, and (3) immunization of susceptible strains of mice with peptide fragments of the whole Tg molecule in order to identify regions on the antigen capable of inducing experimental autoimmune thyroiditis.

3.1. Linear Epitopes on Tg Determined by Analysis of Peptide Fragments of the Molecule

Several workers have analyzed the reactivity of anti-Tg antibodies with peptide fragments of the Tg molecule. Shepherd and colleagues (24) have digested human Tg by staphylococcal V8 protease and produced a variety of fragments with molecular weights between 10,000 and 460,000. Six of the fragments demonstrated heterogeneous binding of 24 HT sera. However, the anti-

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bodies bound more efficiently to the larger fragments, suggesting that they recognized conformational epitopes (24). One disadvantage of using digestion fragments of Tg is that the sequences of the fragments are unknown. In order to overcome this problem and to establish the exact localization and sequence of linear epitopes on the human Tg molecule, Henry *et al.* (25) produced fusion proteins containing human Tg peptide fragments by inserting the corresponding cDNA in an expression vector. Using rabbit polyclonal anti-Tg antibodies, they characterized 7 epitopes on human Tg. Five of the epitopes are concentrated in the median region of the molecule. When tested with these peptide fragments, the immunoreactivities of sera from nine HT, four Graves' disease (GD), and two thyroid carcinoma patients were highly heterologous, even for sera from different patients with the same pathology, and no relationship could be found between the pathology and the epitope pattern (25). Similarly, Dong *et al.* (26) have defined 10 epitopes of Tg by screening a λ gt11 human thyroid cDNA library with heterologous anti-Tg antibodies. However, sera from HT patients did not bind to any of the 10 defined heteroepitopes. Recently, Saboori *et al.* (27) have reported that Tg from two Graves' thyroids contained low levels of organified iodine compared to Tg from three normal thyroids. Additionally, sera from five thyroiditis patients showed greater binding to one fraction of Graves' Tg than to the corresponding fraction of normal Tg. These findings may suggest that there are antigenic differences between Tg from normal and Graves' thyroids and that anti-Tg antibodies from patients with AITD react differently to these different forms of Tg (28).

3.2. Mapping Conformational Epitopes on Thyroglobulin by Inhibition of Binding of Monoclonal Anti-Tg Antibodies

The chief weakness of studies of antibody reactivity to Tg peptide fragments is that they enable identification only of linear epitopes. In order to study conformational epitopes on the Tg molecule, several workers have performed inhibition studies utilizing panels of anti-Tg monoclonal antibodies. Ruf *et al.* (29) identified six reactivity groups using a panel of murine anti-human Tg monoclonal antibodies. Interestingly, none of these reactivity epitopes contained iodinated tyrosines. Anti-Tg antibodies pooled from sera of 48 patients with GD reacted with one of the six clusters, thus suggesting that anti-Tg antibodies in GD recognize unique determinants on Tg (29). By cross-inhibition with 15 anti-human Tg monoclonal antibodies, Piechaczyk *et al.* (30) detected six clusters of reactivity reflecting six antigenic domains on the Tg molecule (31). In their studies too, sera from patients with thyroiditis reacted mainly with one domain (cluster II), whereas anti-Tg antibodies

from healthy individuals bound to other regions in a less restricted fashion (30). Consequently, Henry *et al.* (32) used the same panel of 15 monoclonal antibodies to screen protease-digest products of human Tg and human Tg cDNA expression libraries. By this approach they were able to localize exactly the Tg determinant of cluster II. Of the 15 anti-human Tg monoclonal antibodies only 4 recognized a low molecular weight digested peptide of human Tg and a recombinant epitope expressed in human Tg cDNA libraries. After subcloning, this antigenic determinant was reduced to a 102-amino-acid peptide (human Tg region of amino acids 1149–1250), and this epitope was the one most frequently detected by sera from patients with AITD (32).

Kazatchkine and co-workers have reported that anti-Tg autoantibodies from patients with HT express a cross-reactive idiotype termed T44 which is not expressed by natural anti-Tg autoantibodies in the serum of healthy individuals (33). They then determined the epitopic reactivity of various anti-Tg autoantibodies by competitive inhibition assay with a panel of 15 monoclonal antibodies that defines six antigenic clusters on Tg (34). Using this assay it was found that T44-positive autoantibodies from seven patients with HT recognized a specific epitopic cluster (cluster II) on human Tg, yet none of the natural anti-Tg autoantibodies examined recognized this cluster. These studies suggest that most disease-associated anti-Tg autoantibodies are restricted in both their epitopic specificity and their idiotypy, while natural anti-Tg autoantibodies from sera of healthy individuals are polyclonal in their epitopic recognition on Tg (34). The polyclonal nature of anti-Tg autoantibodies from healthy subjects is characteristic of natural autoantibodies (35).

Rose and co-workers have studied extensively the conformational epitopes on Tg by using panels of monoclonal anti-Tg antibodies (4). Since anti-Tg antibodies are found in almost all patients with AITD they tried to examine whether certain Tg epitopes are relevant to the pathogenesis of AITD. They produced a panel of 20 monoclonal anti-thyroglobulin antibodies. Twelve of the twenty monoclonal antibodies reacted with human Tg and not with Tg from other species. Additionally, 3 of the 4 monoclonal antibodies which recognized epitopes widely shared by many mammalian species could be inhibited by T4 and T3, thus suggesting that the hormonogenic regions may be more highly conserved (4). When reciprocal antibody inhibition assays were performed, twelve reactivity groups were distinguished. Three of the twelve reactivity groups had overlapping determinant clusters and were identified as immunodominant. All of the monoclonal antibodies in the immunodominant clusters reacted with human Tg and not with Tg from seven other species tested. The investigators then tested the reactivities of anti-Tg-positive human sera from patients with HT, GD, differ-

entiated thyroid carcinoma, and nontoxic multinodular goiter and from healthy subjects (36, 37). Tg antibodies from HT patients recognized three major clusters on the Tg molecule (clusters II, III, and IV). Similar restriction was observed for GD patients' sera which recognized mainly cluster II. In contrast Tg antibodies of healthy subjects and of patients with nontoxic goiter did not show preferential cluster recognition. Interestingly, anti-Tg antibodies of patients with thyroid carcinoma recognized the same determinants as HT sera (4). Further studies have shown that anti-Tg antibodies from healthy individuals react with phylogenetically conserved epitopes on Tg, whereas anti-Tg antibodies in individuals with autoimmune thyroiditis appear to be directed against more recently evolved epitopes (38). Recently, Prentice *et al.* (39) have also demonstrated that Tg autoantibodies from patients with AITD recognize different antigenic epitopes on Tg than Tg autoantibodies from healthy blood donors. However, they also found that Tg autoantibodies with similar epitopes specificity were derived from different germline genes.

In summary anti-thyroglobulin antibodies from patients with autoimmune thyroiditis have been shown to recognize conformational epitope clusters on Tg in a restricted manner (4), whereas their reactivity to linear epitopes is polyclonal (25). These findings may suggest that all autoepitopes of Tg that are associated with AITD are conformational (26).

3.3. Anti-Tg Antibodies in Experimental Autoimmune Thyroiditis

Experimental autoimmune thyroiditis (EAT) is believed to be a model for human chronic lymphocytic thyroiditis (40). It can develop either spontaneously in certain strains of animals (e.g., an obese strain of chickens) or after immunization with mouse or human thyroglobulin in susceptible mouse strains (i.e., mice bearing H-2^s and H-2^k haplotypes (41)). The disease is characterized by production of anti-thyroglobulin antibodies and lymphocytic infiltration of the thyroid gland and may be accompanied by a reduction of serum thyroid hormone concentrations (42). In order to identify which epitopes on Tg are important in the pathogenesis of EAT and conceivably in human autoimmune thyroiditis, various fragments of the whole Tg molecule have been tested for their ability to induce EAT when injected into susceptible mice. Several determinants on Tg were found to be capable of inducing murine EAT, thus suggesting that they represent immunodominant epitopes on Tg associated with AITD.

Charreire and colleagues have found that tryptic fragments of Tg were able to induce thyroid lymphocytic infiltration in CBA/J mice similar to that observed after immunization with Tg (43). Moreover, the onset and course of EAT correlated with the presence of auto-

antibodies directed against these tryptic fragments of Tg (44). These fragments were isolated and sequenced. One peptide showed significant homology with a portion of the human Tg molecule and was used to synthesize the homologous human Tg peptide called F40D (comprising amino acids 1672 to 1711). The F40D peptide was able to generate thyroid lymphocytic infiltration in CBA/J mice (45); however, no anti-Tg antibodies were produced in the mice. The lack of production of anti-Tg antibodies may suggest that the F40D peptide represents a cryptic epitope on Tg that is not accessible to autoantibodies (46). Additionally, they have shown that injection of a monoclonal anti-idiotypic antibody carrying the internal image of one epitope of the F40D peptide could protect CBA/J mice from further EAT induction by Tg (40). The variable regions of anti-F40D monoclonal antibodies were sequenced and compared with two other anti-Tg monoclonal autoantibodies specific for other Tg epitopes (47). Sequence analysis revealed no restriction in the use of one particular V_H gene segment for the recognition of both Tg and F40D peptide by the monoclonal autoantibodies studied. The lack of substantial homologies in V_H amino acid sequences between the anti-F40D monoclonal autoantibodies does not necessarily mean that they recognize different epitopes on the F40D peptide. It is possible that monoclonal autoantibodies arising from different gene rearrangements are able to adopt similar conformational structures which allow them to recognize the same epitope. Hopefully, more studies examining the variable region gene usage of anti-Tg autoantibodies bearing similar idiotypes will enable us to determine if this indeed is the case.

Carayanniotis and co-workers (48) have used algorithms predicting T cell epitopes within a protein antigen to identify pathogenic Tg sequences. Using this approach they have identified a 17-mer Tg peptide named TgP1 (corresponding to amino acids 2495–2511 of human Tg) that induces experimental autoimmune thyroiditis (EAT) in mice and rats (49, 50). The authors then demonstrated that 5 weeks after priming mice with TgP1 the binding of their IgG with native Tg is completely inhibited by TgP1, suggesting lack of recognition of other epitopes on mouse Tg (51). These results may suggest that the anti-Tg antibodies generated in mice immunized with TgP1 are pathogenic anti-Tg antibodies specific to TgP1, rather than secondary antibodies induced by autologous Tg release from the destroyed thyroid gland (51). Carayanniotis *et al.* (52) have identified another Tg peptide (TgP2, corresponding to amino acids 2695–2713 of human Tg) that causes EAT in SJL mice, but not in other mouse strains. TgP2 appears to identify a T cell epitope since adoptive transfer of TgP2-primed SJL lymph node cells to naive syngeneic recipients induced a pronounced mononuclear infiltration of the thyroid, which was more

extensive than that observed after direct peptide challenge (52).

Thyroglobulin peptides have also been used to study T cell reactivity to epitopes on Tg. Roitt and colleagues (53) have found that two autoreactive murine Tg-specific T cell hybridomas recognized iodination-related epitopes in the Tg molecule, exclusively located in T4-containing peptides. They were able to demonstrate that these Tg reactive T cell hybridomas recognized a 9-mer peptide corresponding to amino acids 2551–2559 of human Tg and containing the T4 at position 2553 (53). The T4 was shown to be a critical residue, since its replacement with other amino acids produced non-stimulatory peptides. T cells reactive with this 9-mer peptide could adoptively transfer EAT to naive recipients (15). Again, the T4 residue was shown to be important in the development of the disease since Tg tryptic peptides depleted of T4 failed to stimulate pathogenic effectors *in vitro*. However, the inability to induce thyroiditis directly by immunization with the T4-containing peptide raises the possibility that other epitopes may be of pathogenic importance.

4. ANTI-IDIOTYPES OF ANTI-THYROGLOBULIN ANTIBODIES

Anti-idiotypes that are complementary to the antigen binding site of the idiotypic which they recognize must be stereochemically similar to the epitope on the antigen which the idiotypic recognizes, i.e., they bear the "internal image" of the antigen (54). The realization that auto-anti-idiotypes are produced during the course of the normal immune response has led to the idiotypic network theory of Jerne (55). Jerne has proposed that the idiotypic–anti-idiotypic interactions function in the regulation of the immune response, either to enhance or to suppress the antibody response to an antigen (55). Indeed, the idiotypic network is believed to constitute one of the arms regulating immune responses leading to suppression or enhancement of the immune response, and examples exist of both suppression and priming by anti-idiotypes (56, 57).

4.1. Anti-Tg Idiotypes in Human Autoimmune Thyroid Diseases

As mentioned earlier anti-Tg antibodies are produced in patients with AITD as well as in healthy individuals (natural anti-Tg antibodies). It is now well documented that natural anti-Tg antibodies and disease-associated anti-Tg antibodies can be distinguished by their differing idiotypic specificities. Matsuyama *et al.* (58) have produced heterologous anti-idiotypic antisera to anti-Tg antibodies in rabbits. They then identified a cross-reactive idiotypic (CRI) in 15 HT patients studied. Similarly, Delves and Roitt (59) produced rabbit anti-idiotypic reagents against three different human auto-

antibodies to Tg. One of the three rabbit anti-sera produced recognized a cross-reactive idiotypic which was expressed in 50% of Hashimoto's patients tested. Kazatchkine and co-workers (33) have also identified a CRI recognized by anti-Tg autoantibodies from patients with HT. This CRI, termed T44, is not expressed by natural anti-Tg autoantibodies in the serum of healthy individuals.

Recently, Delves *et al.* (60) have produced three monoclonal anti-Tg antibodies by fusing Hashimoto's patients' lymphocytes with a mouse myeloma cell line. Rabbit anti-idiotypic sera were produced to each of these monoclonal anti-Tg antibodies and were used to investigate idiotypes on anti-Tg antibodies. They identified a CRI on all three Tg-specific monoclonal anti-Tg antibodies. This CRI appears to involve, at least in part, the binding site for Tg, as increasing concentrations of Tg partially inhibited binding of the anti-idiotypic to the monoclonal antibodies. However, the idiotypic determinants that were observed in these monoclonal antibodies could not be detected on Tg antibodies present in sera from the lymphocyte donors, nor on serum antibodies from other patients with AITD. Since these hybridomas were formed from EB-virus-transformed blood B cells it is possible that the virus activated an anergic B cell to produce antibody not normally secreted in these patients (60).

4.2. Anti-Tg Idiotypes in Experimental Autoimmune Thyroiditis

Zanetti and Bigazzi (61) have studied the effects of anti-idiotypes to anti-rat Tg antibodies in BUF (Buffalo) rats, a strain of rats that develop thyroiditis spontaneously (62). They produced and characterized rabbit anti-idiotypic sera against rat anti-Tg antibodies. These anti-idiotypic antibodies were capable of inhibiting the *in vitro* binding between thyroglobulin and thyroglobulin autoantibodies obtained from BUF rats. Moreover, repeated injection of anti-idiotypic antibodies into sublethally X-irradiated BUF rats with autoimmune thyroiditis was followed by a significant decrease in the levels of circulating anti-Tg autoantibodies when compared to controls.

Charreire and colleagues have produced two monoclonal anti-Tg antibodies, one of which (3B8G9) was specific for tryptic fragments of Tg capable of inducing EAT and another which was not specific for these tryptic fragments (43). F(ab')₂ fragments of these monoclonal antibodies were used to determine the levels of anti-idiotypic antibodies to anti-Tg antibodies in CBA/J mice in which EAT was induced by immunization with Tg. According to Jerne's theory of the idiotypic network the anti-idiotypes recognizing F(ab')₂ of the 3B8G9 monoclonal antibody bear the internal image of the thyroideogenic tryptic fragments of Tg. It was found

that the levels of anti-idiotypic antibodies to anti-Tg antibodies not directed against the thyroidogenic epitope were unrelated to EAT activity, whereas the levels of anti-idiotypic antibodies to the monoclonal anti-Tg antibody specific for the thyroidogenic epitope (3B8G9) paralleled with EAT activity (44). These findings suggested that the idiotypes recognizing the thyroidogenic epitope are the pathogenic idiotypes involved in the initiation of EAT in CBA/J mice.

We have recently been able to break tolerance and induce anti-Tg antibodies in BALB/c mice (H-2^d strain of mice, not susceptible to thyroiditis) by idiotypic manipulation (63). The technique of idiotypic manipulation is based on immunizing naive mice with a pathogenic human or mouse autoantibody (i.e., an antibody associated with an autoimmune condition), Ab₁. Consequently, the mice develop anti-autoantibodies (Ab₂), and after 2–3 months anti-anti-autoantibodies (Ab₃), having similar binding characteristics to Ab₁. Since Ab₃ bears the internal image of Ab₁ it can produce in the mice the same pathologic lesions as those associated with Ab₁ (64). We have immunized 15 BALB/c mice with a monoclonal mouse anti-human thyroglobulin (hTg) antibody; controls were immunized with an irrelevant mouse IgG. The mice immunized with anti-hTg antibody developed, 6 weeks after immunization, autoantibodies to human thyroglobulin, but not to dsDNA, cardiolipin, or myeloperoxidase. The presence of specific autoantibodies was associated with low production of thyroid hormones, yet during a follow-up of 20 weeks the mice did not develop characteristic histological signs of thyroiditis. We believe that the anti-Tg antibodies induced in mice by idiotypic dysregulation may have caused the low levels of thyroid hormones without lymphocytic infiltration of the gland. Indeed, it has been shown that anti-Tg antibodies might impair thyroid function in the absence of thyroiditis (65).

5. CONCLUSION: ARE ANTI-Tg AUTOANTIBODIES PATHOGENIC?

One of the key unanswered questions in the study of autoimmune diseases is whether autoantibodies characteristic for an autoimmune condition can indeed induce pathologic changes or whether they are nonpathogenic and are produced following tissue damage and release of autoantigens (i.e., an epiphenomenon). Autoimmune thyroid diseases are characterized by the appearance of anti-Tg antibodies (23). However, whether these autoantibodies are directly involved in the pathogenesis of AITD or are merely produced as a result of an immune response to Tg released from the damaged thyroid is still moot, and data supporting both hypotheses have been produced (Table 1).

Anti-thyroglobulin antibodies were reported in up to 27% of normal individuals (66–70). The presence of

natural anti-Tg antibodies in healthy subjects may suggest that these antibodies are not pathogenic as no thyroid abnormalities are observed in these individuals. However, numerous studies summarized above have clearly shown that the epitope recognition pattern of the natural anti-Tg antibodies observed in healthy subjects is different from that of AITD-associated anti-Tg antibodies. Most studies have demonstrated a restricted epitope recognition pattern by anti-Tg autoantibodies of AITD patients, in contrast to a polyclonal reactivity observed with anti-Tg antibodies from healthy subjects (30, 34, 37). These findings may imply that the anti-Tg antibodies which characterize HT are not produced as a result of a simple immune response to Tg released by the destruction of the thyroid by infiltrating lymphocytes. It can be hypothesized that these oligoclonal antibodies may participate in the initiation of autoimmune thyroiditis. In contrast to the oligoclonal nature of thyroiditis-associated anti-Tg autoantibodies, natural anti-Tg autoantibodies occurring in healthy individuals are not restricted in their epitope recognition and are most probably produced by a polyclonal activation of B cells reactive with Tg (37). The demonstration of catalytic activity of anti-thyroglobulin antibodies, capable of hydrolyzing radiolabeled Tg, may suggest that natural anti-thyroglobulin antibodies may participate in the metabolic clearance of Tg by a mechanism that remains to be defined (71).

Studies on anti-thyroid autoantibodies in pregnancy also support the notion that they are pathogenic autoantibodies rather than merely markers of thyroid damage and release of autoantigens. Several studies have demonstrated that pregnant women with anti-thyroid antibodies in their serum are more likely to develop postpartum thyroiditis, thus suggesting a pathogenic role for these antibodies in initiating the disease (72). Moreover, it was recently reported that pregnant women who were positive for anti-thyroid antibodies (anti-Tg and/or anti-TPO antibodies) had an increased risk of fetal loss in the first trimester, compared with women who were thyroid autoantibody negative (73, 74). These findings lend further support to the notion that certain anti-Tg antibodies bearing specific idiotypes may be pathogenic.

EAT serves as a model for studying pathogenic mechanisms in human autoimmune thyroiditis. The effector mechanisms responsible for the development of EAT are still debatable (2, 75). Abundant data have demonstrated that T cell responses are involved in the induction of the disease (76). Early experiments have shown lack of correlation between anti-Tg antibody levels and the severity of the immunopathological lesions in EAT (77, 78). Moreover EAT was successfully transferred into naive syngeneic recipients by lymphoid cells (79), but not by serum (75). The T cells capable of transferring EAT were later

TABLE 1

Evidence for and against a Role of Anti-Tg Antibodies in the Pathogenesis of Autoimmune Thyroiditis

- I. Evidence against the pathogenic potential of anti-Tg autoantibodies
 1. Anti-Tg antibodies are frequently found in healthy subjects (66–69).
 2. Monoclonal anti-Tg autoantibodies are found in individuals with monoclonal gammopathies without evidence of thyroid abnormalities (70).
 3. The level of anti-Tg antibodies does not correlate with disease activity in human autoimmune thyroiditis and in EAT (77, 78).
 4. In some studies it was not possible to passively transfer EAT by anti-Tg antibodies (75).
- II. Evidence for a pathogenic role of anti-Tg antibodies in autoimmune thyroiditis
 1. AITD-associated anti-Tg antibodies are oligoclonal, in contrast to the polyclonal nature of natural anti-Tg antibodies found in healthy individuals (30, 34, 37).
 2. Anti-Tg antibodies in pregnancy increase the likelihood of development of postpartum thyroiditis and fetal loss (72–74).
 3. In some experiments EAT was passively transferred by anti-Tg antibodies (2, 83–87).
 4. Induction of anti-Tg antibodies by idiotypic manipulation was associated with low levels of thyroid hormones (63).

shown to be cytotoxic T cells (Thy-1⁺ cells in mice) (75, 76). Furthermore, Ly-1⁺ T lymphocyte line cells specifically reactive to Tg that were isolated from mice primed with mouse Tg mediated adoptive transfer of thyroiditis (80). Remy *et al.* (81) demonstrated that cloned cytotoxic T cells could lyse only syngeneic thyroid epithelial cells, but not allogeneic thyroid epithelial cells or syngeneic kidney cells, suggesting the existence of class I-restricted Tg-specific pathogenic cytotoxic T cells. Indeed, recently it was demonstrated by Southern blotting and sequence analysis that intrathyroidal T cells and Tg-specific T cell lines from mice with EAT are restricted in the expression of murine T cell receptor V β genes, suggesting that EAT is associated with oligoclonal expansion of the intrathyroidal T cell populations recognizing Tg (82).

Despite these convincing data pointing to a primary cytotoxic T cell involvement in the pathogenesis of EAT, there are several studies demonstrating passive transfer of EAT by anti-Tg antibodies. In early experiments, Rose and co-workers (83) were able to passively transfer thyroiditis by serum from animals immunized with thyroid antigen. Similarly, Nakamura and Weigle (84) reported successful transfer of thyroid lesions in rabbits by sera from rabbits immunized with Tg, and by purified anti-Tg antibodies from the same donors. Similar results were reported in other studies in mice (85, 86) and in the obese strain (OS) of chickens (87). Mice implanted with anti-Tg antibody-secreting hybridomas develop thyroid lesions (88), and anti-Tg antibodies were shown to impair thyroid function in the absence of thyroiditis (65). Additionally, there is some evidence that anti-Tg antibodies may be implicated in the destruction of the thyroid gland (89). Recently it was shown that *in situ* perfusion of rabbit thyroids with high-titered homologous sera reactive with saline thyroid extract has led to thyroiditis (2). These results may suggest that EAT can be passively transferred by anti-Tg antibodies only if the thyroid is exposed to

high levels of the antibodies. If this is indeed the case, it may explain the discrepancies in the results of previous reports on passive transfer of EAT by anti-Tg antibodies. Additionally, in our experiments we found that anti-Tg antibodies induced in mice by idiotypic dysregulation were associated with low levels of thyroid hormones without lymphocytic infiltration of the gland (63).

In summary, the data presented demonstrate that anti-Tg antibodies in AITD are directed toward a restricted number of epitopes on Tg which are different than the epitopes recognized by anti-Tg antibodies found in healthy individuals. These epitopes are mainly conformational, and they are most probably located in the nonhormonogenic regions of the molecule which are much less conserved evolutionally than the hormonogenic sites. Although traditionally it is believed that AITD are T-cell-mediated diseases, there is growing evidence to suggest that anti-Tg antibodies may play a role in the initiation of autoimmune thyroiditis, albeit the full-blown disease can develop only if specific cytotoxic T cells are generated.

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Received April 8, 1996; accepted July 5, 1996