Unique Autoantibody Epitopes in an Immunodominant Region of Thyroid Peroxidase*

(Received for publication, October 20, 1995, and in revised form, December 21, 1995)

Patricia L. Arscott, Ronald J. Koenig, Michael M. Kaplant, Gary D. Glick, and James R. Baker, Jr.

From the Departments of Internal Medicine and Chemistry, University of Michigan, Ann Arbor, Michigan 48109

To define the autoantibody epitopes in amino acids 513–633 of thyroid peroxidase (TPO), a region frequently recognized in thyroiditis, cDNA sequences coding for peptide fragments of this region were amplified and ligated into pMalCRI and pGEX vectors for expression as recombinant fusion proteins. Western blots and enzyme-linked immunosorbent assay were then used to examine the reactivity in sera from 45 Hashimoto’s disease and 47 Graves’ disease patients. Two autoantibody epitopes within TPO amino acids 589–633 were identified; 16 of 35 patients reactive to TPO513–633 recognized the epitope of TPO592–613, while 6 patients recognized the epitope of TPO607–633. Eleven other patients with thyroiditis and two with Graves’ disease recognized only the whole 589–633 fragment, and this response accounted for the Hashimoto’s disease specificity. An amino acid sequence comparison of TPO592–613 with analogous regions of other peroxidase enzymes revealed significant differences in this area, and the substitution of even a single amino acid in one of the epitopes markedly decreased the binding affinity of autoantibodies. Additionally, the exclusive recognition by patients of only one of the epitopes within this region suggests a genetic restriction of the autoantibody response.

Thyroid peroxidase (TPO) is an autoantigen that is recognized by autoantibodies from patients with either Hashimoto’s thyroiditis or Graves’ disease (1). However, the basis of immune responses to this antigen in autoimmune thyroid disease (AITD) is not clear (2, 3). Studies have indicated that TPO is a complex autoantigen having at least two conformational and several localized autoantibody epitopes (4–6). Two TPO regions, amino acids 513–633 and 710–740, have been identified as containing autoantibody binding sites (6, 7), and we have attempted to correlate autoantibodies to these regions with manifestations of AITD (8). No difference was observed in the overall serologic response to either native or denatured TPO in Graves’ disease and Hashimoto’s thyroiditis (8). However, autoantibodies against TPO amino acids 513–633 were identified more commonly in Hashimoto’s thyroiditis patients than in Graves’ disease patients (8). This has focused our current studies on the antigenic characterization of this region of TPO.

The exact nature of the autoantibody epitope or epitopes in TPO amino acids 513–633 is not clear. The location and conformational dependence of epitopes in this region may be important because this area contains a cysteine which may be involved in intra- or interchain disulfide linkages in the TPO enzyme complex (9). A histidine residue proposed to be involved in tethering the heme coenzyme is also located within this site, suggesting a role in enzymatic function (10). This region of TPO shares significant structural homology with myeloperoxidase (11) and lactoperoxidase (12), suggesting the possibility of antigenic cross-reactivity or molecular mimicry (13, 14). This suggests that there may be unique structural and immunologic aspects to this area of TPO. Therefore, characterizing the antigenicity of this region might lead to an understanding of the potential role of this activity in disease pathogenesis (15).

In order to better understand the antigenicity of this region, we employed recombinant fusion proteins and synthetic peptides to characterize the autoantibody epitopes within the region of TPO amino acids 513–633. The structural nature and specificity of these sites were clarified, and the associations with different forms of autoimmune thyroid disease were analyzed. The results suggest that the autoantibody responses to this region in patients with AITD are heterogeneous in nature and may be genetically restricted.

MATERIALS AND METHODS

Patient Sera—Serum from 45 patients with Hashimoto’s disease, 47 patients with Graves’ disease, and 34 age- and sex-matched normal controls were studied. The age of the patients ranged from 17 to 82 years. The Hashimoto’s patients were diagnosed on the basis of antimicrosomal antibody titers greater than or equal to 1:400 by agglutination assay in the presence of either goiter or hyperthyroidism. Graves’ disease was diagnosed on the basis of clinical and biochemical evidence of hyperthyroidism (suppressed TSH with elevated T4), diffuse goiter, and an increased 24-h radioiodine uptake. None of the controls had clinical evidence or a history of thyroid disease.

Clinical Measurements—The thyroid functions tests (including FT4, FT3) were performed in a routine clinical laboratory, and the TSH levels were determined by a chemiluminescence immunosassay with a sensitivity of 0.05 milliunits/liter (Magic-Lite, Corning, Medfield, MA). Thyroglobulin and microsomal antibody titers were determined by agglutination assay (Ames Diagnostics, Ames, IA). Goiter size and the presence of ophthalmopathy were determined by one of the investigators (M. K.) who was unaware of the TPO reactivity of the subjects.

Construction of TPO Expression Plasmids Using PCR—Oligonucleotides were designed and used in various combinations as PCR primers to generate cDNAs coding for TPO fragments within the region of amino acids 513–633 (Fig. 1). The primers were designed so that the resulting PCR products contained an EcoRI recognition sequence at the 5’ end, followed by the (in-frame) TPO coding sequence, a stop codon, and an XbaI recognition site at the 3’ end. Plasmid DNA from pMalTPO513-

* This work was supported by NIAID, National Institutes of Health Grant AI 37141-01. Core support was obtained from Grant P60 DK20572–16. This work was presented in abstract format at the National Meeting of the American Federation of Clinical Research. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† To whom all correspondence should be addressed: Dept. of Internal Medicine and Pathology, 1150 W. Medical Center Dr., 1520 MSRB I, University of Michigan Medical School, Ann Arbor, MI 48109-0666.

§ This abbreviation is defined in “Abbreviations.”

¶ This abbreviation is defined in “Abbreviations.”

||

4966
Autoantibody Epitopes in Thyroid Peroxidase

633 developed from pMalRl expression plasmid (New England Biolabs) containing (in-frame) the cDNA coding for TPO amino acids 513–633 (8) was used as a template (10–50 ng/mixture) with 200 ng of the 5′ and 3′ primer, 0.8 mM mixed dNTP, 4 mM MgCl2, 1.25 units of Taq DNA polymerase in 50 mM KCl, 10 mM Tris-HCl, pH 9.0 with 0.1% Triton X-100 (Promega) in a total volume of 50 μl. The reaction was carried out with pMalRl and GST protein, produced from bacteria transformed with vector without TPO insert, were also electrophoresed and blotted as control antigens.

TPO Fragment ELISA—A MAP peptide (17) consisting of TPO amino acids 592–613 coupled to a polylysine backbone was synthesized (University of Michigan Protein Core Facility). The peptide was first solubilized in dimethyl sulfoxide and then diluted to 0.05 mM sodium carbonate, pH 9.6, buffer with 0.02% sodium azide. Immunol-2 ELISA plates (Dynatech) were coated by adding 100 μl/well and incubating overnight at 4°C. The wells were then washed with PBS-A with 0.05% Tween-20 and blocked with 150 μl/well of 10% nonfat dry milk in PBS-A and incubated for 1 h at 27°C. After washing the wells, patient serum was diluted in PBS-A containing 10% nonfat dry milk and added in serial dilutions from 1:200–1:25,600 at 100 μl/well and incubated for 2 h at 37°C. The wells again were washed, and then incubated for 1 h at 37°C with 100 μl/well of alkaline phosphatase-conjugated goat anti-human IgG (J Jackson Laboratories, West Grove, PA) diluted at 1:5000 in PBS-A. Controls for the specific reactivity to the TPO fragment was confirmed by dideoxynucleotide sequencing spanning the 5′ insertion site of the TPO cDNA sequence. Clones from each construct were grown and induced to produce fusion protein and then purified as described (7). Each protein was then characterized by Western blotting using a mouse monoclonal antibody to detect the TPO fusion proteins, and to human sera previously found to be reactive to the 513–633 fragment, to detect specific reactivity to the TPO fragment.

Construction of TPO59–613 Containing Substituted Amino Acids—Oligonucleotides were synthesized to correspond to TPO59–613 cDNA sequence that altered glutamic acid residues at positions 593 and 596 in TPO. Two sense strand oligonucleotides were designed for amino acids 589–604. One of the strands coded for serine and glycine residues at position 593 and 596, respectively, corresponding to the amino acid at the homologous positions in lactoperoxidase (LPO) (5′-AATTCCTGAGGTAGACTAGATGGCTGTGCTCAGGTCAGGGGGTTAGACTAGATGGCTGTGCTCAGGTCAGGGGCCCAAGGTGATGAGGAGGTTCTGCGGCCTGCCTCGCCGGTTAGACTAGATGGCTGTGCTCAGGTCAGGG) and the other sense strand oligonucleotide coded for alanine at amino acid 593 and arginine at amino acid 596, corresponding to the amino acids at positions 589–604. One of the strands coded for serine and glycine residues at position 593 and 596, respectively, corresponding to the amino acid at the homologous positions in lactoperoxidase (LPO) (5′-AATTCCTGAAGGCTGGTTGGTGCTGGCCGTTGGCTGAGGAGGTTAGACTAGATGGCTGTGCTCAGGTCAGGGGCCCAAGGTGATGAGGAGGTTCTGCGGCCTGCCTCGCCGGTTAGACTAGATGGCTGTGCTCAGGTCAGGG). Both sequences maintained the TPO coding sequence for all other residues within the 589–613 region. A common antisense strand was synthesized to match the TPO cDNA sequence for amino acids 592–613 (8). GST-TPO fusion proteins were also confirmed by Western blot using a polyclonal mouse serum to GST (produced in our core facility by immunization with the GST portion of the fusion protein). Blocking possible antibody reactivity to the GST portion of the fusion protein, sera were preincubated with 0.1 mg/ml purified recombinant GST protein for 1 h at 27°C before being added to the wells.

Inhibition of ELISA Using TPO Fusion Proteins—Binding of serum antibodies to the TPO592–613 peptide by ELISA was inhibited by the various amino acid containing amino acids from within the 513–633 region. Serum from eight patients positive to the TPO592–613 epitope was diluted with 10% milk/PBS-A to 1:400 and 1:1,000. Inhibitor concentrations from 10–5 to 10–12 M were preincubated with the diluted serum for 2 h at 27°C. The mixture was then added to the diluted serum to 1:200–1:25,600 and included as an internal control on each plate, providing a standard for comparison. ELISA using GST fusion proteins was also performed using the sequences TPO598–633 or TPO607–633 to coat plates at 10 μg/ml. Serum reactivity was assayed as described with the peptide. To block possible antibody reactivity to the GST portion of the fusion protein, sera were preincubated with 0.1 mg/ml purified recombinant GST protein for 1 h at 27°C before being added to the wells.

Construction of Epitope Structure—Computational molecular modeling was conducted on a Silicon Graphics 360GT computer running the ISIGHT/DISCOVER software package. Epitopes were constructed in a turn geometry as suggested by the mapping experiments, and conformational energy was minimized using a conjugate-gradient algorithm and the AMBER united atom force field to a first derivative of 1.0 kcal/mol/Å2. Structure predictions for the TPO peptide used the Karplus neural net algorithm as implemented in the PROTEIN PREDICTOR software package (18). The results from the analysis were compared with predictions using the Kyle-Dodich and Chothia routines to ensure appropriateness (19). Analysis of Amino Acid Sequence Similarities—Amino acid sequence analysis for the specific epitopes within this region was conducted as a BLASTA search through the National Library of Medicine (20). RESULTS

Mapping of TPO513–633 Fragment Epitope Using Truncated Fusion Proteins—PCR amplification of the TPO cDNA and ligation into pMalRl produced a number of different fusion proteins with sequences corresponding to TPO amino acids within the region 513–633, as depicted in Fig. 1. These MBP-TPO fusion proteins were initially screened by Western blots for reactivity using sera from Hashimoto’s patients with high titer antibody to amino acids 513–633 (8). Positive sera were diluted to 1:400 and did not demonstrate binding to the MBP-LacZ control protein at this dilution. Western blots of fusion proteins produced by the different constructs, incubated with a monoclonal antibody to the MBP portion of the fusion protein (Fig. 2, A and C, left lanes), document all of the MBP-TPO fusion proteins and indicate that equivalent amounts of all of the protein are present in both transfers (also demonstrated by

Downloaded from http://www.jbc.org/ on April 26, 2019

4967

The End-Blocked TPO592–613 peptide was also confirmed by Western blot using a polyclonal mouse serum to GST (produced in our core facility by immunization with the GST portion of the fusion protein). Blocking possible antibody reactivity to the GST portion of the fusion protein, sera were preincubated with 0.1 mg/ml purified recombinant GST protein for 1 h at 27°C before being added to the wells.

Inhibition of ELISA Using TPO Fusion Proteins—Binding of serum antibodies to the TPO592–613 peptide by ELISA was inhibited by the various amino acid containing amino acids from within the 513–633 region. Serum from eight patients positive to the TPO592–613 epitope was diluted with 10% milk/PBS-A to 1:400 and 1:1,000. Inhibitor concentrations from 10–5 to 10–12 M were preincubated with the diluted serum for 2 h at 27°C. The mixture was then added to the diluted serum to 1:200–1:25,600 and included as an internal control on each plate, providing a standard for comparison. ELISA using GST fusion proteins was also performed using the sequences TPO598–633 or TPO607–633 to coat plates at 10 μg/ml. Serum reactivity was assayed as described with the peptide. To block possible antibody reactivity to the GST portion of the fusion protein, sera were preincubated with 0.1 mg/ml purified recombinant GST protein for 1 h at 27°C before being added to the wells.

Construction of Epitope Structure—Computational molecular modeling was conducted on a Silicon Graphics 360GT computer running the ISIGHT/DISCOVER software package. Epitopes were constructed in a turn geometry as suggested by the mapping experiments, and conformational energy was minimized using a conjugate-gradient algorithm and the AMBER united atom force field to a first derivative of 1.0 kcal/mol/Å2. Structure predictions for the TPO peptide used the Karplus neural net algorithm as implemented in the PROTEIN PREDICTOR software package (18). The results from the analysis were compared with predictions using the Kyle-Dodich and Chothia routines to ensure appropriateness (19). Analysis of Amino Acid Sequence Similarities—Amino acid sequence analysis for the specific epitopes within this region was conducted as a BLASTA search through the National Library of Medicine (20). RESULTS

Mapping of TPO513–633 Fragment Epitope Using Truncated Fusion Proteins—PCR amplification of the TPO cDNA and ligation into pMalRl produced a number of different fusion proteins with sequences corresponding to TPO amino acids within the region 513–633, as depicted in Fig. 1. These MBP-TPO fusion proteins were initially screened by Western blots for reactivity using sera from Hashimoto’s patients with high titer antibody to amino acids 513–633 (8). Positive sera were diluted to 1:400 and did not demonstrate binding to the MBP-LacZ control protein at this dilution. Western blots of fusion proteins produced by the different constructs, incubated with a monoclonal antibody to the MBP portion of the fusion protein (Fig. 2, A and C, left lanes), document all of the MBP-TPO fusion proteins and indicate that equivalent amounts of all of the protein are present in both transfers (also demonstrated by
protein stain of gel, Fig. 2B). Blots with a Hashimoto's disease serum (Fig. 2, A and C, right lanes) demonstrate positive and negative binding where the patient's serum recognizes the fusion protein band from pMalTPO592–613 but not from pMalTPO589–607. All of the different sized TPO fusion proteins were similarly tested for reactivity using patient sera and showed definitive positive or negative binding using this technique. Employing the fragments outlined in Fig. 1, three different epitopes were suggested as being present in this region of TPO; one epitope corresponding to the amino acids from 592 to 613 showed reactivity with the highest number of patients and shared significant sequence homology with analogous regions of MPO and LPO (Fig. 3).

To determine whether patients reacted to more than a single epitope within the larger region of amino acids 513–633, lysates of the various MBP-TPO fusion proteins were used to inhibit binding to MBP-TPO513–633 on Western blot (Fig. 4). Serum from 5 positive patients and the anti-MBP monoclonal antibody were preincubated with either 1% bovine serum albumin (“+” strips), MBP-TPO592–613 lysate (A strips), MBP-TPO607–633 lysate (C strips), or purified MBP-TPO513–633 (B strips), then incubated with strips from a Western blot of MBP-TPO513–633. Control strips incubated with anti-MBP monoclonal antibody identify the 56-kDa MBP-TPO513–633 fusion protein and confirm that preincubation with the other MBP fusion proteins would inhibit antibody binding. Sixteen patients in all demonstrated binding to the TPO592–613 fragment and had the reactivity to the TPO513–633 fusion protein inhibited by preincubation with this fragment (“A” strips, demonstrated by the blot from patient H4), while in 13 other patients, binding to the TPO513–633 fusion protein was not inhibited by either or both smaller fusion proteins (demonstrated by the blot from patient H3). GST-TPO fusion proteins containing the same TPO amino acid sequences (Fig. 2D) demonstrated identical patterns of reactivity to what was observed with the MBP-TPO fusion proteins, indicating that the fusion protein or linker portion of the construct did not alter the binding to this region of TPO and was not involved in the inhibition. The ability of autoantibodies against TPO513–633 to bind whole native TPO was assessed by Western blot using affinity-purified antibody. Affinity-purified antibody to 513–633 recognized only the reduced form of native TPO (data not shown).

**Patient Reactivity to Epitopes within TPO Amino Acids 513–633—**To examine the frequency of autoantibodies to these various regions in patients with Graves' disease and Hashimoto's thyroiditis, reactivity to either TPO592–613, TPO607–633, or TPO589–633 fragments was measured by ELISA and Western blot (Table I). Patient reactivity to TPO592–613 also was measured by ELISA using a MAP peptide corresponding to this sequence. Patient sera were analyzed at serial dilutions from 1:200–1:25,600; representative dilution curves for 6 positive and 5 negative patients are presented in Fig. 5. Absorbances from either 1:400 or 1:800 serum dilutions were compared to the dilution curve for the positive control, and relative value units were calculated for each patient. Positive patients were identified by both the relative units and the antibody titer, the latter being defined as the lowest serum dilution maintaining activity above background. No normal serum demonstrated binding to this peptide (0 of 30). 8 of 45 Hashimoto's thyroiditis patients and 9 of 47 Graves' disease patients were positive to
the TPO592–613 epitope. Positive antibody titers ranged from 1:200 to 1:25,600; however, there was no correlation between these values and the overall titers of microsomal antibodies as determined by agglutination assay. Among the reactive Hashimoto’s thyroiditis patients, the average titer to TPO592–613 was 1:9,450 while the reactive Graves’ disease patients had an average titer of 1:7,475. (Median titers for Graves’ disease patients were 1:1,600 and 1:3,200 for Hashimoto's thyroiditis patients).

ELISA employing GST fusion proteins containing TPO589–633 or TPO607–633 were used to examine the patients who reacted with other epitopes within TPO513–633. 4 of 31 Hashimoto’s thyroiditis patients and 2 of 25 Graves’ disease patients tested were positive to the 607–633 epitope. Thirteen sera that bound TPO513–633 also reacted with the whole 589–633 region, but not to either the 592–613 or the 607–633 epitope, reinforcing the finding of the inhibition study that this whole fragment appeared to be a single epitope in some patients. Patients reactive to TPO589–633 but not to TPO592–613 also did not recognize TPO589–613. 34 of the 35 patients with autoantibodies reactive to the TPO589–633 region exclusively recognized one of the defined epitopes (Table I).

Affinity of Autoantibodies to TPO592–613—Competitive inhibition of serum reactivity to the TPO592–613 peptide ELISA using different concentrations of TPO fusion proteins was employed to provide a relative estimation of antibody affinity. Affinity was defined as the concentration of inhibitor reducing ELISA reactivity of the serum by 50%. Overlapping GST-TPO fusion proteins including the sequence of amino acids 592–613 were used to inhibit serum from eight patients with high titer antibody to this epitope. Fig. 6 shows representative ELISA inhibition curves from a positive patient. Preincubation of pos-
The presence of autoantibodies to localized regions of TPO within amino acids 513–633 as determined by ELISA and Western blot. The amino acid sequence of TPO592–613—substitutions within 592–613—substitutions at positions 595 and 602, possibly stabilize the loop via charge linkages with other portions of the molecule. The charged residues at 601 and 606 (Fig. 8). The cysteine at position 598, near the "tip" of the loop, appears to be available for disulfide linkages with other portions of the molecule. The charged residues, glutamic acids at positions 593 and 596-glycine (as in LPO), and the construct containing the single 596-glycine substitution, each increased the amount of fusion protein required to inhibit binding to 592–613 by 10–100-fold. Although many TPO592–613 reactive patients demonstrated some reactivity with the 593-serine and 596-glycine-substituted (LPO) constructs in this assay, the apparent affinity was in all cases less than what was seen with the TPO construct (ranging from no binding to 4 \times 10^{-6} M). Structural Modeling of the Region—Modeling of the region from amino acids 592 to 613 did not demonstrate secondary structure of either \( \beta \) sheet or \( \alpha \) helical configuration. Instead, the region appeared to be a semirigid loop defined by proline residues at 598 and 601 (Fig. 8). The cysteine at position 598, near the "tip" of the loop, appears to be available for disulfide linkages with other portions of the molecule. The charged residues, glutamic acids at positions 593 and 596, and the arginine at positions 595 and 602, possibly stabilize the loop via charge interaction.

Clinical Associations—Although TPO592–613 autoantibodies appear in a large number (15 of 35) of TPO513–633 reactive patients, there was no apparent relationship between Hashimoto's thyroiditis and autoantibody binding. As seen in Table I, differences in binding in Hashimoto's and Graves' disease patients seem to be accounted for by antibodies reactive only with the whole region of amino acids 589–633; 11 thyroiditis patients (24%) reacted to this region as compared to only 2 Graves' disease patients. There was no correlation between overall TPO reactivity in patients reactive to any of these epitopes. Anti-thyroglobulin antibodies were found in 85% of
the patients studied and also did not correlate with reactivity to any localized epitope.

No correlation between reactivity to 592–613 and measures of thyroid function such as FT4, FT3, TSH levels, iodine uptake, or presence of goiter was observed for either Hashimoto's thyroiditis or Graves' disease patients. However, among the thyroiditis patients, hypothyroidism was significantly more common in those that recognized TPO592–613 (7 of 8, 88%) as compared to individuals recognizing the whole 589–633 epitope (5/11, 45%, \( p < 0.05 \)).

DISCUSSION

These studies identify three autoantibody epitopes in the region defined by TPO amino acids 589–633; two containing only portions of the amino acid sequence, as well as the whole region which was also recognized as a single epitope. Computer modeling of this area revealed neither α helix nor β sheet structure, but instead the region appears to be a loop held in conformation by prolines at positions 601 and 606. A cysteine at residue 598, just before the two prolines and near the tip of the loop, suggests that the region may be involved in disulfide linkages within the TPO molecule. This is in accordance with the structure of TPO, which is currently believed to be a disulfide-linked dimer of two 105-kDa chains containing both intrachain and interchain disulfide bonds (9, 10, 21, 22). Autoantibodies against the 589–633 region are unable to bind to TPO unless it is reduced, indicating that the epitopes are inaccessibly in the native molecule. Together, this information indicates that the epitopes within the 589–633 region of TPO may be neotopes, or sites that are not normally exposed to the immune system (23).

The serologic response to this particular region of TPO provides a number of insights into immune mechanisms underlying autoimmune thyroid disease. The autoantibody response to TPO is heterogeneous with different individuals recognizing unique epitopes. This has also been observed with the immune response to conformational epitopes in TPO, and it has been hypothesized to occur due to differences in immunoglobulin variable region genes that might allow interaction with specific...
autoantibody binding sites (24). In contrast to the response to conformational epitopes, the immune response to epitopes in the 589–633 region is restricted to a single site in almost all the individuals examined. The basis of this mutually exclusive epitope recognition is not clear, but it is possible that several of the binding sites within this region are initially recognized, and the immune response then evolves to a single, strongest epitope that excludes the other responses. This possibility is difficult to evaluate since the patients we investigated had mature autoimmune disease and had already evolved their autoantibody response. This restricted recognition could also be based on a genetic restriction, not the result of evolutionary changes in the immune response, and the evaluation of TPO epitope recognition in relatives of these patients may help to clarify this issue.

While portions of the 589–633 region demonstrate substantial conservation in amino acid sequence with analogous areas of other peroxidase enzymes, including myeloperoxidase and lactoperoxidase (11, 12), areas of the amino acid sequences of the autoantibody epitopes within this region appear to be unique to TPO. Particularly, the charged residues and the prolines are unique to TPO and appear necessary to maintain antigenicity. Substitution of one of the charged amino acids with the corresponding sequence of LPO or MPO markedly decreases antibody affinity for the most dominant epitope in this region. This suggests that TPO autoantibodies develop from recognition of the autoantigen and not molecular mimicry from LPO or other antigens. However, a cross-reactive response to another antigen, such as LPO, that has subsequently affinity matured specifically to the autoantigen, cannot be excluded entirely since these patients have well-established autoimmune disease.

The importance of antibodies binding to epitopes within the 589–633 region in the diagnosis and treatment of AITD is of interest. The titer of antibodies directed against this particular region of TPO varies between different individuals and does not correlate with the overall anti-TPO titer. As a result, some patients have high titer antibody against this region but relatively modest titers of anti-TPO antibodies in conventional assays. This has the practical implication that anti-TPO titers in some patients may be underestimated with serologic assays that measure antibodies against native TPO. The mechanisms underlying the recognition of this site in AITD is difficult to ascertain. It is possible that these epitopes are recognized only after inflammation has disrupted thyroid follicular cells and released unfolded or reduced TPO, a form of epitope spreading. Recognition of the whole region, which is restricted to Hashimoto’s disease patients, would seem likely to be the result of this process. However, the presence of autoantibodies against the local epitopes did not correlate with overall anti-TPO titers, a putative marker of glandular inflammation and release of TPO. This makes it less likely that the response to these epitope is based purely on this process. The presence of antibodies to the 592–613 epitope in patients with Graves’ disease, which is not associated with extensive thyroid follicular cell disruption, would also seem to argue against this. However, this latter finding might be explained by an overlap syndrome, where thyroiditis and Graves’ disease exist concurrently. Prospective studies screening for TPO epitope autoantibodies in Graves’ disease may therefore identify individuals who have concurrent thyroiditis and might not need thyroid ablative therapy.

Other autoimmune diseases have identified heterogeneous responses to autoantigens, particularly in inflammatory processes. The autoantibody response to the acetylcholine receptor
in myasthenia gravis is similarly diverse, with the recognition of both localized and conformational epitopes on the α chain of this protein (25). The recognition of glutamic acid decarboxylase in insulin-dependent diabetes mellitus is also heterogeneous with recognition of both localized and conformational binding sites (26). It has been difficult in both of these situations to associate any particular manifestation of the disease with variations in autoantibody epitope recognition, so the lack of an association in this case is not surprising. Graves’ disease has been suggested to be associated with several types of autoantibodies to the TSH receptor protein (27). However, responses to particular epitopes in the TSH receptor have not been entirely characterized (28), and mutually exclusive epitope recognition, in a manner similar to the response against the 589–633 region of TPO, has not been described. Thus, the findings of the present work on TPO in AITD support and extend what has been observed in other autoimmune diseases.

In conclusion, these studies demonstrate conclusively that the autoimmune response to TPO in autoimmune thyroid disease is heterogeneous and appears restricted in most patients and indicate that the initial events of TPO recognition involve the autoantigen itself. However, multiple factors are likely to be involved in initiating the autoimmune response in thyroiditis and Graves’ disease, and some of these may be related to environmental antigens. These variables, together with the patient’s own genetic background, might best account for the heterogeneity in immune responses that are seen in this protein in these diseases. Monitoring the response to the epitopes of TPO in the 589–633 area over time in patients with autoimmune thyroid diseases may clarify the evolution of the autoantibody response in these disorders and provide a better understanding of how the immune response to this protein evolves and contributes to thyroid disease pathogenesis.
Unique Autoantibody Epitopes in an Immunodominant Region of Thyroid Peroxidase
Patricia L. Arscott, Ronald J. Koenig, Michael M. Kaplan, Gary D. Glick and James R. Jr. Baker

J. Biol. Chem. 1996, 271:4966-4973.
doi: 10.1074/jbc.271.9.4966

Access the most updated version of this article at http://www.jbc.org/content/271/9/4966

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 28 references, 1 of which can be accessed free at http://www.jbc.org/content/271/9/4966.full.html#ref-list-1