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The Thyroxine-Containing Thyroglobulin Peptide (aa 2549–2560) Is a Target Epitope in Iodide-Accelerated Spontaneous Autoimmune Thyroiditis

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Enhanced iodide ingestion is known to accelerate the incidence and severity of spontaneous autoimmune thyroiditis [iodide-accelerated spontaneous autoimmune thyroiditis (ISAT)] in NOD.H2h4 mice. CD4+ cells are required for the development and maintenance of ISAT, but their target epitopes remain unknown. In this study, we show that the previously identified thyroglobulin (Tg) T cell epitope p2549–2560 containing thyroxine at position 2553 (T4p2553) induces thyroiditis as well as strong specific T and B cell responses in NOD.H2h4 mice. In ISAT, activated CD4+ T cells specific for T4p2553 are detected before the disease onset in thyroid-draining cervical lymph nodes only in mice placed on an iodide-rich diet and not in age-matched controls. In addition, selective enrichment of CD4+ IFN-γ+ T4p2553-specific cells is observed among cervical lymph node cells and intrathyroidal lymphocytes. T4p2553 was equally detectable on dendritic cells obtained ex vivo from cervical lymph node cells of NaI-fed or control mice, suggesting that the iodide-rich diet contributes to the activation of autoreactive cells rather than the generation of the autoantigenic epitope. Furthermore, spontaneous T4p2553-specific IgG are not detectable within the strong Tg-specific autoantibody response. To our knowledge, these data identify for the first time a Tg T cell epitope as a spontaneous target in ISAT. The Journal of Immunology, 2014, 193: 96–101.

NOD.H2h4 mice are well known to develop spontaneous autoimmune thyroiditis (SAT) at a much higher incidence than the H2-congenic NOD strain (1–5), while remaining free of diabetes (6). In this model, addition of 0.05% NaI in the drinking water has been found to accelerate both the incidence and the severity of SAT (1–4, 7–10), providing opportunities to study the interplay of genetics and environment in the progression of autoimmune disease. Iodide-accelerated spontaneous autoimmune thyroiditis (ISAT) is characterized by mononuclear cell infiltration of the thyroid and strong serum IgM and IgG responses to thyroglobulin (Tg) (3, 4, 7–10). Immunohistochemical staining studies have suggested that the thyroid is initially infiltrated by CD4+ and CD8+ cells (11–13), and depletion experiments have shown that CD4+ cells are required for the development and maintenance of ISAT (3, 4). Weak proliferative responses of thyroid-draining cervical lymph node cells (cLNCs) to Tg have been observed at various time points after the initiation of the NaI diet regimen (10), but the target epitopes recognized by CD4+ T cells, during the initial stages of the autoreactive cascade in ISAT, remain unknown. Because in NOD.H2h4 mice (Kk, Aβ, E0, Dβ) functional MHC class II Ags are limited only to Aβ molecules, such epitopes are expected to be recognized by Aβ-restricted CD4+ T cells.

Tg is the most abundant protein in the thyroid gland and encompasses many Aβ-restricted T cell epitopes, which can elicit experimental autoimmune thyroiditis (EAT) when administered into mice with adjuvant (14). In NOD.H2h4 mice developing ISAT, we have not been previously able to detect proliferative splenic cell responses to several Aβ-restricted pathogenic T cell epitopes, 56 d following the initiation of iodide supplementation (10). To address the concern that thyroid Ag–specific T cells may not be detectable in the periphery during later stages of the disease because they might have already migrated into the thyroid, we have in this study examined Ag-specific responses of cLNCs in the initial stages of ISAT and of intrathyroidal lymphocytes during the course of the disease. Our attention was focused on the Aβ-restricted Tg peptide p2549–2560 containing thyroxine at position 2553 (T4p2553) because it can induce lymphocytic (15) as well as granulomatous EAT (16) and can be seen as a target by class I–restricted cytotoxic T cells in vitro (17). Furthermore, T4p2553-specific T cell clones can be activated in vivo following mouse challenge with intact Tg (15). The results show that T4p2553 can be immunopathogenic in NOD.H2h4 mice and comprises a target epitope in ISAT recognized by spontaneously emerging CD4+ T cells.

Materials and Methods

**Animals**

NOD.H2h4 mice were bred and maintained in the animal facility of the Faculty of Medicine, Memorial University of Newfoundland, St. John’s, NL, Canada. Both male and female NOD.H2h4 mice were used in groups that were age and sex matched. All experimental procedures were performed in accordance with the Canadian Council on Animal Care and the guidelines of the Animal Care Committee of the Faculty of Medicine, Memorial University of Newfoundland.

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reviewed and approved by the Animal Care Committee at Memorial University of Newfoundland.

**Ags**

Tg was purified from thyroid glands of female CD-1 mice (Charles River, Quebec, Canada) after centrifugation of thyroid extract at 16,000 × g, followed by passage through a Sepharose CL-4B column (Amersham Biosciences, Uppsala, Sweden), as previously described (10). F-moc t-thyroxine was produced as previously described (18, 19). The 12-mer Tg peptide Tg4p2553 (aa 2549–2560), STDXT4T5SFR4AL, was synthesized at the University of Patras, Greece, and at Biosynthesis (Lewisville, TX) at >90% purity. The 19-mer OVA peptide (322–340), CISVQAHHAAEL-NEAGRY, was synthesized at the Alberta Peptide Institute (Edmonton, AB, Canada) at >80% purity. Both peptides were blocked with an acetyl group at the N terminus and with an amide group at the C terminus. OVA was purchased from Sigma. CFA (with Mycobacterium butyricum) andIFA were purchased from Difco (Detroit, MI).

**Culture media and lymphocyte proliferation assays**

Inguinal, brachial, and axillary LNCs from Ag-primed mice were cultured in DEMEM supplemented with 10% FCS (PAA Laboratories, Etobicoke, ON, Canada). In ISAT, thyroid-draining LNCs or spleen cells were cultured in HL-1 medium (Lonza, Walkersville, MD). Media were supplemented with 100 U/ml penicillin/streptomycin, 2 mM glutamine (Life Technologies, Invitrogen, Grand Island, NY), and 5 × 10^−5 M 2-ME (Sigma-Aldrich, St. Louis, MO). RPMI 1640 (Life Technologies, Invitrogen) with 10% FCS was used in activation assays of T cell hybrids. Lymphocyte proliferation assays were performed by culturing 4 × 10^5 cells per well with Ag in 96-well flat-bottom plates for 3–4 d at 37°C. During the last 1 h, 1 μCi [3H]thymidine (PerkinElmer, Boston, MA) was added to each well. Cells were harvested in a Classic Cell Harvester (Skatron Instruments), and radioactivity was measured in a LS6500 Multi-Purpose Scintillation Counter. Stimulation index (S.I.) was defined as cpm in the presence of Ag/cpm in the absence of Ag.

**Generation and characterization of the KCl hybridoma clone**

LNCs from Tg4p2553-primed NOD.H2b mice were activated in vitro with 20 μM Tg4p2553 for 4 d and were subsequently fused with BW5147 α-β cells (20), kindly provided by P. Marrack (National Jewish Medical and Research Center, Denver, CO), using polyethylene glycol 1500 (Roche Diagnostics, Mannheim, Germany). Screening for IL-2–secreting, peptide-specific hybrids was done using Tg3 cells as APCs and the IL-2–dependent CTLL line, as previously described (21). Cloning was performed by limiting dilution at 0.3 cell per well. The IgG2a mAbs specific for Ag (TIB 92) or influenza A nucleoprotein (NP) (HB 65) (American Type Culture Collection, Manassas, VA) were purified from culture supernatants, as previously described (22). Percent inhibition of KCl activation by blocking mAbs was calculated as follows: [% 1/cpm (in the presence of mAb)/cpm (in the absence of mAb)] × 100.

**Isolation of dendritic cells and intrathyroidal lymphocytes**

To obtain dendritic cells (DCs), spleens and/or cervical lymph nodes were digested with Liberase TM Research Grade (Roche Applied Science, Mannheim, Germany). Isolation of dendritic cells and intrathyroidal lymphocytes

**Statistical analysis**

The nonparametric Mann–Whitney U test was used to assess statistical significance between mouse groups developing ISAT. The one-tailed unpaired Student t test was applied for other comparisons, using GraphPad Prism 4 software.

**Results**

**Tg4p2553 is an immunopathogenic epitope in the NOD.H2b strain**

To examine the immunogenicity of Tg4p2553 in NOD.H2b, 6- to 8-wk-old mice were s.c. challenged with 100 nmol of peptide in CFA. Nine days later, inguinal, brachial, and axillary LNCs were pooled and tested for recall responses against various Ags in vitro. Strong proliferative LNC responses were detected in the presence of 1 μM (S.I. = 9) and 10 μM of Tg4p2553 (S.I. = 15), whereas background responses were obtained against Tg or OVA (Fig. 1A). When NOD.H2b mice were challenged with 100 μg of Tg in CFA for 9 d, weak (S.I. = 2–3) but significant proliferative LNC responses were observed against Tg4p2553, but the response to Tg itself or OVA remained at background levels (Fig. 1B). To assess the pathogenicity of Tg4p2553, 8-wk-old NOD.H2b mice (n = 10) were primed with 100 nmol Tg4p2553 in CFA for 3 wk and further boosted with 50 nmol of the same peptide in IFA for 2 wk. Control mice (n = 8) were similarly sensitized with 50 μg and boosted with 25 μg OVA. Histological examination of the thyroids showed mononuclear cell infiltration in 10 of 10 mice of the experimental group (mean I.I. = 2.1), which was significantly higher than that observed in only 2 of 8 OVA-challenged controls (mean I.I. = 0.38) (Fig. 1C, 1D). The collective data demonstrated the immunopathogenic properties of the Tg4p2553 T cell epitope in

**Measurement of IgG responses by ELISA**

Spontaneous IgG responses were monitored in pooled mouse sera by ELISA, as previously described (10). Briefly, wells were coated with 10 μg/ml Tg or 10 μg/ml OVA or 80–100 μg/ml T4p2443 in carbonate buffer, pH of 9.6 overnight, washed with PBS, and blocked with 0.1% BSA in PBS for 1 h at room temperature. The plates were then washed, and sera, diluted in PBS/Tween 20 containing 0.1% BSA, were added to wells for 1 h. After an additional wash, an alkaline phosphatase–conjugated goat anti-mouse IgG Ab (Jackson ImmunoResearch Laboratories) was added to wells for 1 h, followed by addition of the p-nitrophenyl phosphate substrate (Sigma-Aldrich). Light absorption was determined at 405 nm, using a VMax plate reader (Molecular Devices, Sunnyvale, CA).

**ISAT development and EAT induction in NOD.H2b mice**

To initiate ISAT, 6- to 8-wk-old mice were placed on drinking water containing 0.05% NaCl, and thyroiditis development and/or autoreactive BAs and T cell responses were monitored at various time points thereafter. To induce EAT, NOD.H2b mice were s.c. challenged with 100 nmol Tg4p2553 peptide in CFA emulsion, and 3 wk later they were boosted with 50 nmol of the same peptide in IFA. EAT was assessed 5 wk after the initial challenge. Thyroids were fixed in 10% formalin, embedded in methacrylate, step sectioned, and stained by H&E, as previously described (24). Mononuclear cell infiltration of the thyroid was scored as follows: 0, no infiltration; 1, interstitial accumulation of cells between two or more follicles; 2, one or two foci of cells at least the size of one follicle; 3, diffuse infiltration of 10–40% of the total area; 4, extensive infiltration of 40–80% of the total area; 5, extensive infiltration of >80% of the total area. Data represent the highest infiltration index (I.I.) observed within each gland.

**Intracellular cytokine staining**

CD4+ cells were purified from cLNCs and/or spleens using a mouse CD4+ T cell enrichment kit (StemCell Technologies, Vancouver, BC, Canada). Subsequently, they were cultured at 2 × 10^5 cells per well in HL-1 medium with splenic DCs (4 × 10^5 cells per well) and Ag in 96 flat-bottom wells for 4 d. During the last 6 h, 5 μg/ml Brefeldin A (Sigma-Aldrich) was added to the wells. All staining steps were performed at 4°C. FCRs were blocked with anti-mouse CD16/32 (clone 93; eBiosciences, San Diego, CA) for 15 min and subsequently incubated with an FITC-conjugated anti-CD4 Ab or isotype control (BD Biosciences, Mississauga, ON, Canada) for 30 min. After three washing steps, cells were fixed in 2% paraformaldehyde and permeabilized with 0.5% saponin in PBS + 2% FCS. Then, staining with a PE-conjugated anti-mouse IFN-γ and the isotype control mAb was performed (BD Biosciences) for 30 min at 4°C.

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NOD.H2h4 mice during a time interval (8–13 wk) in which SAT is minimally present.

**Accumulation of T4p2553-specific T cells in thyroid-draining cLNCs and in the thyroid**

Next, we proceeded to determine whether T4p2553-specific T cells could be detected within thyroid-draining cLNCs before the ISAT onset. NOD.H2h4 mice were placed on an NaI/H2O diet for 7, 11, and 15 d, and recall in vitro assays against T4p2553 were performed at each time point, using cLNCs or splenic cells. As shown in Fig. 2A, strong cLNC responses (S.I. = 8) against T4p2553, but not against Tg or OVA, were detected after 11 d of NaI intake, whereas the cLNC response from control mice placed on a regular diet remained at background levels. Day 7 or day 15 responses to any of the Ags tested were undetectable (data not shown). Furthermore, day 11 T4p2553-specific responses of splenocytes were weak and not significantly different between the experimental and control groups (Fig. 2B), indicating selective enrichment of Ag-specific T cells in the thyroid-draining lymph nodes and not in the periphery.

The presence of activated T4p2553-specific T cells in cLNCs of iodide-fed mice was further examined by intracellular cytokine staining 12 d after the diet initiation. CD4+ T cells were purified from cLNCs and cultured with splenic DCs as a source of APCs, in the presence of Ag. Culture with T4p2553 activated a significantly higher number of IFN-γ−producing cells from cLNCs of iodide-fed mice versus the controls (13.21% versus 6.22%) (Fig. 2C). This difference was not evident in cultures containing the OVA peptide (322–340) as an Ag control (Fig. 2C). In contrast to cLNC-derived cells, the percentage of splenic CD4+ IFN-γ+ cells responding to either peptide did not differ significantly between iodide-fed and control mice (Fig. 2D). Finally, 35 d after the initiation of the dietary regimen, intracellular cytokine staining of thyroid-infiltrating lymphocytes showed a small but significant difference in the percentage of CD4+ IFN-γ+ intrathyroidal lymphocytes responding to T4p2553 versus OVA peptide (10.22% versus 7%) (Fig. 2E). Overall, these data strongly suggested that an iodide-rich diet led to an early enrichment of activated T4p2553-specific cells in cLNCs as well as in lymphocytic infiltrates of the thyroid during ISAT development.
**FIGURE 3.** T4p2553 is constitutively present on DCs from cLNCs. (A) T4p2553-specific reactivity of the KC1 hybridoma clone cultured (10^5 per well) with the Ags shown and TA3 cells (10^3 per well) as APCs. Activation was measured by IL-2 secretion and the use of the IL-2-dependent line, CTLL. (B) Inhibition of KC1 activation by blocking mAbs specific for A^k or NP (at 10 μg/ml) in the presence of 25 nM of T4p2553 and TA3 cells as APCs. Data show the mean ± SEM of triplicate wells and are representative of two experiments. (C) Detection of T4p2553 on CD11c^+ cells purified from cLNCs of iodide-fed or control mice, 12 d after initiation of the iodide-rich diet. Ex vivo obtained CD11c^+ cells (1 × 10^6 per well) were cultured with KC1 (5 × 10^4 per well) for 24 h, and activation was assessed by a CTLL proliferation assay. Addition of exogenous T4p2553 yielded ~45,000 cpm. Data represent the mean ± SEM from four to six wells per group and are representative of four experiments. *p < 0.001, **p = 0.003.

T4p2553 is detected on DCs from thyroid-draining cLNCs

To test whether enhanced iodide intake would promote generation of T4p2553 on DCs in lymphoid tissue, we generated KC1, a T4p2553-specific, IL-2-secreting T cell hybridoma clone, as a detection tool. The KC1 clone was selected for its sensitivity, as it responded to ≥12 nM T4p2553 in culture but, interestingly, remained unresponsive to intact Tg in the 100- to 200-nM range (Fig. 3A). KC1 activation was strongly inhibited by an A^k-specific mAb, but not by a control mAb specific for influenza NP, confirming presentation of T4p2553 in the context of the A^k molecule (Fig. 3B). A low but significant activation of KC1 was equally detectable following its coculture with ex vivo obtained DCs from NaI-fed or control mice, 12 d after initiation of the dietary regimen (Fig. 3C). These data suggested a constitutive presence of T4p2553 on DCs in thyroid-draining cLNCs. Coculture of KC1 with increasing numbers of ex vivo obtained DCs (from 5:1 up to 1:1 KC1/DC ratio) did not induce stronger KC1 activation (data not shown), indicating that the amount of T4p2553 on DCs is very small.

In ISAT, T4p2553-specific B cells do not contribute significantly to the Tg autoantibody repertoire

Following challenge with T4p2553, 6- to 8-wk-old NOD.H2^bd mice mounted strong peptide-specific as well as Tg-specific serum IgG responses, as assayed by ELISA (Fig. 4A), suggesting localization of the T4p2553 epitope on the surface of the Tg molecule. The response was not due to spontaneous production of anti-Tg IgG because anti-Tg or anti-T4p2553 IgG responses were not detected in OVA-challenged, age-matched mice (data not shown). In contrast, in mice developing ISAT, T4p2553-specific serological IgG responses were not detected after 30, 40, or 60 d on the NaI/water diet despite the presence of anti-Tg IgG abs at those time intervals. (Fig. 4B–D). These results indicated that, in NOD.H2^bd mice developing ISAT, T4p2553-specific IgG does not constitute a detectable portion of the Tg autoantibody repertoire.

Discussion

Our findings show that direct challenge of NOD.H2^bd mice with the Tg peptide T4p2553 generates EAT with 100% incidence and significant severity (mean I.I. = 2.1). Previous studies described no EAT induction after direct challenge of CBA/J mice with T4p2553 (19) or weak peptide stimulation of human Tg-primed lymphocytes from DR3-transgenic NOD mice (25) that are known to develop ISAT (26). In CBA/J mice, EAT induction was observed only after adoptive transfer of T4p2553-specific T cells (15, 16, 19). These discrepancies perhaps reflect differences in the immunization protocols (dose and timing of administered peptide) as well as the mouse strain used. In this study, in vivo priming with intact Tg or Tg peptide allowed for detection of subsequent T4p2553-specific, proliferative LNC responses in vitro, confirming earlier findings by other groups (15, 16, 19). In that regard, it is surprising that deliberate challenge of NOD.H2^bd mice with intact Tg in CFA does not yield detectable recall responses to Tg itself. This has also been observed with 52-wk-old NOD.H2^bd mice, that, at a time when SAT is fully developed (unpublished observations), whereas weak splenic Tg-specific T cell responses are obtained in ISAT (days 28 and 42 of the dietary regimen) at a time when a strong spontaneous Tg-specific IgG response is clearly evident (10). These results are reminiscent of observations in diabetes, in which insulin-specific T cell responses are difficult to detect in NOD mice, either spontaneously or after priming with insulin (27–29), despite the presence of anti-insulin autoantibodies (30). Unresponsiveness to the intact antigen in this system has been attributed to possible generation of suppressor cells mediating dominant tolerance (31). It is noteworthy that T4p2553-specific T cells are unlikely to undergo negative selection pressure in the thymus because thymic cells do

**FIGURE 4.** Screening for IgG responses against T4p2553 in EAT and ISAT by ELISA. Pooled sera from T4p2553-primed NOD.H2^bd mice (n = 10) (A), or NOD.H2^bd mice, placed on an iodide-rich diet for (B) 30, (C) 40, and (D) 60 d (n = 6 per group), were tested for the presence of Tg-specific or T4p2553-specific IgG. Control mice (n = 6 per group) in (B), (C), and (D) received a normal diet. Results are expressed as mean O.D. values of triplicate wells ± SEM after subtraction of background values from wells with no serum added.
not synthesize T4, although they express truncated mRNA isoforms of Tg that encode this peptide sequence (32). The thyroxine moiety with the four bulky iodine atoms forms an integral part of the A^ptide T4p2553 complex because anti-T4 Abs are known to block T cell recognition of this peptide (33).

In this study, we report for the first time, to our knowledge, that a defined Tg epitope is a natural target of the spontaneous T cell response in ISAT. Our previous inability to detect spontaneous splenic T cell responses to known pathogenic Tg peptides in later stages of ISAT (10) prompted us to search for autoreactive T cells in thyroid-draining lymph nodes in early stages of the disease, that is, before day 15 of the NaI/water dietary regimen, when the first signs of ISAT appear in our colony. We observed selective enrichment of T4p2553-specific, proliferative or CD4^+ IFN-"g" T cells in cLNCs—but not in the spleen—only in mice receiving NaI for 11–12 d. Proliferative cLNC responses were undetectable at earlier time points (day 7) probably owing to the low frequency of the peptide-specific T cells, and at later time points (day 15), perhaps owing to their exiting to cLNCs and homing to the thyroid. Early detection of activated autoreactive T cells in cLNCs is analogous to that seen in diabetes, in which CD4^+ and CD8^+ T cell priming in pancreatic lymph nodes occurs before the onset of insulin antibodies in NOD mice (34–36). The presence of T4p2553-specific T cells within thyroidal infiltrates also suggests recognition of this peptide ligand on thyrocytes, which are known to upregulate MHC class II expression after IFN-"g" treatment (37), or peptide recognition on intrathyroidal resident DCs (38).

The detection of T4p2553 on DCs obtained ex vivo from cLNCs of either iodide-fed or control mice suggests their constitutive presence on APCs that is independent of iodide intake. We have previously hypothesized that T4p2553 and several other defined Tg peptides with pathogenic potential constitute part of a “cryptic self” that must be intrathyroidally expressed on resident DCs under steady-state conditions because they are recognized in naive animals by preactivated specific effecter T cells that home to the thyroid and initiate tissue damage (39). Detection of T4p2553 on DCs from cLNCs suggests that either intrathyroidal DCs carrying this peptide migrate to cLNCs or the peptide is transferred to cLNCs through the lymphatic drainage. This process may contribute to peripheral tolerance mechanisms that inactivate autoreactive T cells through anergy or active suppression (39). Analogous findings have been reported in diabetes by Unanue’s group (40), in which CD11c^+ cells from pancreas or pancreatic lymph node were shown to constitutively present hen egg lysozyme (HEL) peptides in mice expressing the HEL gene under the insulin promoter. Constitutive expression of tissue-specific Ags, such as the H^+2-K^+/ATPase protein, (41) or foreign Ags, such as the SV40 T Ag (42), the influenza virus hemagglutinin (43), or OVA (44), by DCs in draining lymph nodes has been proposed to promote peripheral tolerance (45).

Our findings indicate that high iodide intake does not promote T cell autoreactivity to T4p2553 through enhanced generation of this peptide on DCs. Analogous data have been reported with an HEL peptide–specific T cell hybridoma that was equally activated by DCs from normal and from streptozotocin-treated IP-HEL mice (40, 46). Furthermore, in the NOD system it has been previously shown that B cell death, either induced by streptozotocin or transferred by injections of dead cells in the pancreas, promotes activation of the transgenic B1D2.5 CD4^+ and 8.3 CD8^+ T cells in pancreatic lymph nodes (36, 47). Because NOD.H2^b thyrocytes are highly sensitive to iodide-mediated apoptosis (48), it can be argued that apoptosis could trigger autoreactivity by inducing tolerogenic DCs to adopt an immunogenic phenotype, as has been shown in other systems (49).

At the B cell level, T4p2553 priming induced strong peptide-specific IgG responses in NOD.H2^b mice, as previously seen in the CBA/J strain (19). Other groups have reported low or undetectable immunogenicity of T4p2553 in CBA hosts (15, 16), perhaps reflecting dose and route of administration differences in peptide delivery. The strong anti-Tg responses observed in T4p2553-primed NOD.H2^b mice were not detected in age-matched mice challenged with OVA, indicating that they are not spontaneously generated, but rather reflect access of the T4p2553 site on the surface of the Tg molecule by peptide-specific IgG. In view of this, the lack of spontaneous IgG response to T4p2553 in ISAT was surprising, demonstrating that existing T4p2553-specific B cell clones do not contribute to the Tg autoantibody repertoire that characterizes ISAT. This finding suggests that the Tg-specific IgG response is driven against other immunodominant B cell epitopes or by Tg fragments that lack the T4p2553 sequence. Nevertheless, T4p2553-specific B cells could bind to and internalize intact Tg, playing an APC role, as previously suggested in the NOD.H2^b model (50).

In conclusion, this study identifies a Tg T cell epitope as a spontaneous target in ISAT. It also highlights the fact that iodine, as a dietary accelerator that accelerates an autoimmune process, becomes itself an integral part of a target self-epitope recognized by pathogenic T cells. Our ability to activate and monitor autoreactive T cells of a defined specificity provides a new read-out system to study effects of immune intervention in this animal model. It also generates new opportunities for the construction of TCR-transgenic mice in this model, which will be useful in studies of immunoregulation.

Disclosures

The authors have no financial conflicts of interest.

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