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Human thyroglobulin peptide p2340 induces autoimmune thyroiditis in HLA-DR3 transgenic mice

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Abstract

In a previous study we demonstrated that the human thyroglobulin (hTg) peptide p2340 (aa 2340–2359) can stimulate a T cell response and elicit experimental autoimmune thyroiditis (EAT) in AKR/J (H-2^k) mice. In the present study we examined whether p2340 can induce EAT in single HLA class II DR3 transgenic mice.

This peptide was found to be immunogenic at the T cell level in DR3 mice, since it induced specific proliferative responses, as well as IL-2 and IFN-γ secretion in secondary cultures of peptide-primed lymph node cells (LNC). Immunization of HLA-DR3 mice with p2340 in CFA elicited EAT (infiltration index of 1 to 2) in eight of nine mice. Peptide-primed LNC responded to intact hTg, whereas, hTg-primed LNC did not respond to p2340 in culture, suggesting that p2340 contains subdominant T cell epitope(s). P2340 was also found to be immunogenic at the B cell level, since strong p2340-specific IgG response was detected in all transgenic mice tested. Thus, we provide evidence for a pathogenic role of an hTg peptide in HLA-DR3 transgenic mice.

Therefore, p2340 could be presented by DR3 molecule in patients with Hashimoto's thyroiditis and participate in the development of the disease.

Keywords: Experimental autoimmune thyroiditis; HLA-DR3 transgenic mice; T cell epitope; Thyroglobulin

1. Introduction

Thyroglobulin (Tg) is the most abundant protein of the thyroid gland and the largest autoantigen known [1,2]. Mice challenged with Tg or Tg peptides develop experimental autoimmune thyroiditis (EAT), a T cell mediated disease that resembles Hashimoto's thyroiditis (HT) in humans [3–5]. Work from various laboratories over the last 10 years has identified 13 immunopathogenic Tg T cell epitopes [5–8]. Eight of them have been identified in mouse Tg (mTg), three in human Tg (hTg) and two, sharing identical amino acid sequence between mTg and hTg (contain the hormonogenic sites of Tg). In this study, we focused on hTg 20-mer peptide p2340 (aa 2340–2359) which was initially shown to be a target of Tg-reactive autoantibodies occurring in Tg/acetylcholinesterase reactive sera from patients with Graves' disease [9]. Furthermore, p2340 was found to be able to elicit E^k-restricted thyroiditis in AKR/J (H-2^k) mice. This finding raises the possibility that the hTg-derived p2340 may be presented by HLA-DR molecules, which are homologous to the mouse I-E antigens [7]. DR3 allele is associated with numerous autoimmune diseases and is linked to EAT and immunization of DR3 transgenic mice with intact human or mouse Tg induces

Abbreviations: EAT, experimental autoimmune thyroiditis; hTg, human thyroglobulin; LNC, lymph node cell (s); HT, Hashimoto's thyroiditis; CFA, complete Freund's adjuvant; IFA, incomplete Freund's adjuvant; I.I., infiltration index.

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EAT [10–12]. Poor knowledge is available about hTg peptide-induced EAT in these mice; a 15-mer peptide (hTg2079) was recently described to possess pathogenic potential in EAT when administered in DR3 transgenic mice [13].

The presence of three HLA-DR3 binding motifs within p2340 and our previous findings showing pathogenicity of p2340 in AKR/J mice [7], prompted us to examine whether p2340 could elicit T cells and thyroiditis in HLA-DR3 transgenic mice devoid of mouse class II molecules. These mice provide powerful tools to understand the role of HLA class II molecules in predisposition and onset of human thyroiditis and to develop immunotherapy and vaccines [13–16].

2. Materials and methods

2.1. Mice

Transgenic mice deficient in the mouse MHC class II molecules bearing only a human DR ($A\beta^0$.DR3) molecule and (functional DR3 (HLA-DRA1*0101/DRB1*0301) genes) in the C57BL/10 background were selected for this study. All the transgenic mice were obtained after the tenth backcross generation to C57BL/10 mice. In all the transgenic mice, expression of HLA class II and absence of endogenous MHC class II molecules were verified by FACS and PCR [10]. The percentage of CD4+ and CD8+ cells in the peripheral blood leucocytes of DR3 mice was analysed before immunization by flow cytometry [17]. The results showed that the immune system in HLA transgenic mice developed normally, with appropriate expression and functionality of MHC class II molecules. Transgenic mice were initially generated in Dr. Chella David's laboratory and bred at the Mayo Clinic, and then bred and housed in the viral Ab-free barrier facility at the University of Texas Medical Branch. This work was approved by the UTMB Animal Care and Use Committee and the Hellenic Pasteur Institute's Animal House.

2.2. Antigens

The hTg peptide QVAALTWVQTHIRGFGGDPR (aa 2340–2359, p2340) was synthesized by Sigma Genosys (The Woodlands, TX, USA) and had its N- and C-terminals blocked with an acetyl and amide group, respectively. The hTg peptide PYEFSRKVPT FATPWPDFVP (aa 2652–2671, p2652) was synthesized by Genosys Biotechnologies (Cambridge, UK) and used as control. Its properties are previously described [9]. Both peptides were used in all experiments at >80% purity as determined by analytical HPLC and mass spectral analyses.

2.3. Lymph node cell (LNC) proliferation assays

Mice were subcutaneously (s.c.) immunized at the base of the tail with 100 μg hTg or 200 μg (100 nmol) p2340 emulsified in complete Freund's adjuvant (CFA) (Sigma Chemical Co., St. Louis, MO). Ten days later, the inguinal lymph nodes were collected aseptically and single cell suspensions were prepared in DMEM (Gibco, Burlington, Ontario, Canada) supplemented with 10% FBS (Bioproducts for Science, Indianapolis, IN, USA), 20 mM HEPES buffer, L-glutamine, penicillin–streptomycin (all from Gibco) and 5×10^{-5} M 2-ME (Sigma). After centrifugation and washing, cells (4×10^5/200 μl/well) were cultured in the presence or absence of hTg, p2340 and control peptide in flat-bottomed 96-well microculture plates (Costar, Corning Inc., New York) and incubated for 4 days at 37 °C in 5% CO_2, 95% air-humidified incubator. At 18 h before harvesting, 1 μCi of [^{3}H]TdR (25 Ci/mmol, product code TRK-120, Amersham Biosciences, UK) was added to each well in 25 μl of medium. The cells were harvested using a semi-automatic cell harvester (Skatron, Inc., Sterling, VA) and counted in a liquid scintillation counter (LS3801, Beckman Instruments, Inc., Mississauga, Ontario, Canada). Stimulation index is defined as: cpm in the presence of Ag/cpm in the absence of Ag.

2.4. Cytokine detection by enzyme-linked immunosorbent assay (ELISA)

Sandwich ELISA was used for the measurement of cytokines in culture supernatants and based on non-competing pairs of monoclonal antibodies for capture and detection (biotinylated) of anti-cytokine mAbs (IL-2, IFN-γ and IL-10; PharMingen, San Diego, CA). P2340-primed LNC (2×10^6 cells/well) were seeded onto 48-well flat-bottomed culture plates in 1.0 ml of DMEM with and without p2340 or the control p2652. Plates were coated with 5 μg/ml of anti-IFN-γ, IL-10 and IL-2 antibodies overnight at 4 °C. Plates were blocked with 10% FBS in PBS for 2 h at room temperature. Supernatant samples or serially diluted recombinant cytokines (as standards) were added and incubated overnight at 4 °C. Alkaline-phosphatase-conjugated streptavidin was purchased from Sigma. Standard curves were generated for each individual cytokine tested using known amounts of rIL-2, rIFN-γ and rIL-10 (PharMingen). The absorbance (optical density, O.D.) of the produced colour at 405 nm (O.D. 405 nm) was measured using the ELISA reader from Molecular Devices Corp. (Sunnyvale, CA).

2.5. Detection of IgG anti-p2340 activity by ELISA

Wells of polyvinyl chloride microtiter plates (Costar, Corning Inc., New York) were coated overnight at 4 °C