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Research paper

A radioligand binding assay to measure anti-thyroperoxidase autoantibodies in mice

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Abstract

Autoimmune (Hashimoto's) thyroiditis is a chronic inflammatory disease which affects >3% of the population and shows an increasing prevalence with increasing age. Anti-thyroid autoantibodies, particularly against thyroperoxidase (also known as thyroid peroxidase or TPO), occur commonly in humans with autoimmune thyroid disease, and assays for anti-TPO autoantibodies are used in clinical diagnosis. In contrast anti-TPO autoantibodies have not been observed in classical mouse models of autoimmune thyroiditis, except in cases where mice were deliberately immunized with TPO. In the past, detection of anti-TPO autoantibodies in mice has relied on an indirect immunofluorescence assay (iIFA) which screens for thyroid follicle membrane staining in frozen sections of mouse thyroid glands. Since recent transgenic mouse models of autoimmune thyroiditis spontaneously develop anti-TPO autoantibodies, an assay other than serial dilution and iIFA would be useful to detect and quantify these autoantibodies. In this paper we describe such an assay, based on the capacity of autoimmune mouse sera to bind to the extracellular domain of mouse TPO which was produced in a radioactively labeled form using a coupled *in vitro* transcription/translation system. The same approach, using human TPO, could provide a highly sensitive method to detect anti-TPO autoantibodies in humans.

Keywords: Thyroiditis; Autoantibodies; Mouse thyroperoxidase; Radioligand binding assay; Indirect immunofluorescence

1. Introduction

Hashimoto's thyroiditis, also called autoimmune thyroiditis, is a chronic inflammatory disease which leads to thyroid destruction and can lead to a deficiency in thyroid hormone production. Autoimmune thyroiditis occurs in more than 3% of the population and symptoms include fatigue, weight gain and, in some cases, a gradual enlargement of the thyroid gland (goiter). Currently, there is no known intervention that can prevent the autoimmune process leading to Hashimoto's thyroiditis, but hypothyroidism, the end result of the thyroid gland

Abbreviations: bp, base pair(s); BSA, bovine serum albumin; cpm, counts per minute; DEPC, diethylpyrocarbonate; ELISA, enzyme-linked immunosorbent assay; GAD, glutamic acid decarboxylase; hu, human; IA-2, islet antigen 2 (a tyrosine phosphatase); IAA, insulin autoantibodies; ICA, islet cell antigen; iIFA, indirect immunofluorescence; mu, murine; NOD, Nonobese Diabetic; O.C.T., Optimal Cutting Temperature; PBS, phosphate buffered saline; PBS-T, PBS+0.1%; Tween 20; RBA, radioligand binding assay; SDS, sodium dodecylsulphate; TCA, trichloroacetic acid; TSH, thyroid stimulating hormone; TPO, thyroperoxidase; Vt, total volume.

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destruction, can be treated by lifelong thyroid hormone replacement. (Mariotti and Pinna, 2003)

In humans, laboratory tests for both serum thyroid hormone levels and anti-thyroid autoantibodies are routinely performed to assist in the diagnosis of the hypothyroidism induced by Hashimoto's thyroiditis. Hypothyroidism is indicated by normal to low concentrations of circulating thyroid hormones (T3/T4) in the presence of elevated levels of thyroid stimulating hormone (TSH). In addition to changes in hormone levels, autoantibodies against thyroid antigens such as thyroperoxidase (TPO) and thyroglobulin are frequently seen in patients with autoimmune thyroiditis. TPO is a membrane-bound enzyme located on the surface of and in the cytoplasm of thyroid follicular cells; it catalyzes the production of the thyroid hormones T3 and T4 (Mariotti and Pinna, 2003). In humans, anti-TPO autoantibodies have been detected using a variety of assays, including hemagglutination and, more recently, radio- or enzyme-linked- immunoassays.

The development of animal models has enhanced our understanding of autoimmune thyroiditis. However, until recently, none of the known mouse models of autoimmune thyroiditis had shown all the findings of the human disease and, specifically, none had shown spontaneous development of anti-TPO autoantibodies. In fact, one group has questioned whether non-primates are capable of spontaneously developing autoantibodies to thyroperoxidase (Rasooly et al., 1996), even though such antibodies readily appear if mice are immunized with TPO in adjuvant (Ng et al., 2004) or with plasmid or adenoviral vectors (Guo et al., 2003; Flynn et al., 2004).

Currently detection of circulating anti-TPO autoantibodies in mouse sera is accomplished by using an indirect immunofluorescence assay (iIFA) on histological sections of mouse thyroid gland; the assay is positive if a specific pattern of thyroid follicle membrane staining is observed. Although this approach is logical based on the abundance of TPO at this specific anatomical location, the assay is a qualitative rather than a quantitative assay (although serial dilutions can give some indication of titre) and it does not specifically define the thyroid antigen(s) to which the autoantibodies are binding.

In this paper we made use of a transgenic mouse model of spontaneous autoimmune thyroiditis (Hayward et al., manuscript in preparation) which on iIFA clearly develops anti-thyroid follicle membrane autoantibodies. Serum from these animals was used to establish a complementary radioligand binding assay (RBA) for the direct quantification of anti-mouse TPO autoantibodies. Although designed for mouse thyroperoxidase, this type of assay also has the potential to be applied to human thyroperoxidase as an alternative highly sensitive method for diagnosis of anti-thyroid autoimmunity.

2. Materials and methods

2.1. cDNA templates and other reagents

The full-length cDNA encoding murine thyroperoxidase (Kotani et al., 1993), as well as an affinity-purified rabbit anti-human TPO polyclonal antibody known to be cross-reactive with mouse TPO were kindly provded by Dr. K. Umeki (Miyazaki Univeristy, Japan). The portion of the cDNA encoding the extracellular domain (i.e. lacking the predicted N-terminal signal sequence, transmembrane domain, and cytoplasmic segment) was amplified by PCR using Pfu DNA polymerase (Stratagene, La Jolla, CA) and primers CTCTCTATGCATATGAGAAGCAGAGACATCTTGTGT (MuTPO-Excell-NsiI-5′) and CT CTCTCTAGACTAGGATGCCCGAGGTAGCCTG (MuTPO-Excell-XbaI-3′). The resulting ~2450 bp fragment was digested with Nsi I/Xba I and ligated into the vector pcDNAII (Invitrogen, Carlsbad, CA) cut with the same enzymes. The control vectors contained the full-length human GAD65 cDNA in vector pcDNAII and the cytoplasmic segment of human IA-2 (also called 'ICA512bdc') in vector pCRII (Invitrogen); both control vectors were obtained from G. Eisenbarth (University of Colorado Health Sciences Center, Denver, CO). All three vectors contain an upstream SP6 promoter and yield sense strand mRNA in the presence of SP6 RNA polymerase and ribonucleotide triphosphates.

Wash buffer consisted of 150 mM NaCl, 20 mM Tris–HCl pH 7.4, 0.15% (v/v) Tween-20, 0.1% (w/v) BSA, and 0.1% (w/v) sodium azide. The solution was passed through a 0.22 micron filter and stored at 4 °C. Protein G-Sepharose 4B Fast Flow (Sigma #P3296, St. Louis, MO) was washed once with DEPC treated/autoclaved H_2O, twice with wash buffer, then resuspended in wash buffer (50% v/v) and stored at 4 °C.

The mouse B cell hybridoma line GAD6 (produces an IgG2a against human GAD65) was obtained from the Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA; GAD6 monoclonal antibody was affinity purified from hybridoma supernatants using protein G-Sepharose. Rhodamine-conjugated goat anti-mouse IgG antibody (#115-025-062) was purchased from Jackson ImmunoResearch (West Grove, PA).

2.2. Mouse serum

Sera were collected from anaesthetized/euthanized mice via terminal cardiac bleed. The following strains