Low frequency of pendrin autoantibodies detected using a radioligand binding assay in patients with autoimmune thyroid disease

E. Helen Kemp, Harpreet K. Sandhu, Philip F. Watson, and Anthony P. Weetman
Department of Human Metabolism, The Medical School, University of Sheffield, UK

Background and Aim

• Autoimmune thyroid disease (ATD), encompassing autoimmune hypothyroidism (AH) and Graves’ disease (GD), is caused by autoreactivity against several autoantigens: thyroglobulin (TG), thyroid peroxidase (TPO), the thyroid-stimulating hormone receptor (TSHR) and the sodium-iodide symporter. The existence of other autoantigens is possible as autoimmune processes escalate during chronic tissue damage.

• Pendrin is a transmembrane protein located at the apical end of the thyrocyte and mediates iodide efflux through the thyrocyte cell into the colloidal space. Individuals with Pendred syndrome, which results in abrogation of thyroid iodide transport, feature mildly impaired thyroid function.

• Autoantibodies against pendrin have been reported in 81% of ATD patients and 9% of controls using an immunodot blotting method. This technique, however, is largely qualitative in nature and can lack reproducibility of results. In contrast, pendrin autoantibodies could not be detected in ATD using an ELISA or immunofluorescence.

• Given the discrepancy in previous studies, the aim of the present study was to screen a panel of ATD patient sera for pendrin autoantibodies using a novel RBA.

Methods

1. Participants

• 71 unrelated patients (63 female, 8 male; mean age: 37±14 yr) with GD defined by the presence of biochemical hypothyroidism in combination with either: 1) a diffuse goiter on a scan, 2) positive for autoantibodies against TSHR, TG, or TPO, 3) Graves’ ophthalmopathy, or 4) histological confirmation of a lymphocytic infiltrate in thyroid histology. 16 patients were newly diagnosed and untreated, and 55 had been treated with anti-thyroid drugs. Subsequent to anti-thyroid drug treatment, 11 patients had received radio-iodine, three had undergone a thyroideectomy and one had received both radio-iodine and surgery. 12 patients had another autoimmune disease.

• 66 unrelated patients with AH (62 female, 4 male; mean age: 42±14 yr) defined as documented biochemical hypothyroidism and either: 1) positive autoantibodies to TG or TPO, 2) histological confirmation of a lymphocytic infiltrate in thyroid histology, 16 patients were newly diagnosed and untreated, and 55 had received thyroxine replacement. 11 patients had an additional autoimmune disease.

2. Radioligand binding assay for pendrin autoantibodies

• [35] -methionine labelled pendrin was produced from pcDNA3 (contains pendrin) in a T7 T7-Coupled Reticulocyte lysate System (Promega) (Fig. 1a).

• For RBAs, in vitro translated pendrin, equivalent to 100,000 cpm of TCA-precipitable material, was suspended in 50 μl of immunoprecipitation buffer. In duplicate, patient or control sera were added to a final dilution of 1/100. Anti-pendrin antibody sc-16894 (Santa Cruz Biotechnology, Inc.) was a positive control.

• Following overnight incubation at 4°C, 50 μl of protein G Sepharose 4 Fast Flow (GE Healthcare UK, ltd.) were added and incubated for 1 h at 4°C. Protein G Sepharose-antibody complexes were collected by centrifugation and washed extensively. Immunoprecipitated radioactivity was evaluated by scintillation counting.

• A pendrin antibody (Ab) index for each serum was: cpm immunoprecipitated by tested serum/cpm immunoprecipitated by 28 healthy control sera. Each serum was tested in duplicate at 4 and 8 sera concentrations and the mean pendrin Ab index calculated.

Results

1. Radioligand binding assays for autoantibodies against pendrin

• Sera from GD (n=71) and AH (n=66) patients, and healthy controls (n=28) were evaluated for pendrin autoantibodies in RBAs. A pendrin Ab index was determined for each serum sample (Fig 1b).

• Anti-pendrin antibody was included in each assay set as a positive control and displayed a pendrin Ab index of 10.8±1.43.

• The intra- and inter-assay coefficients of variation were 6.0% and 9.2%, respectively.

• The upper limit of normal for the RBA was estimated as a pendrin Ab index of 1.41.

• None of the healthy individuals was positive for autoantibodies against pendrin. For ATD patients, 7/71 (9.9%) of the GD and 5/56 (8.9%) AH patient sera, respectively, were pendrin autoantibodies. Of 16 untreated and 55 treated GD patients, 1 (6.3%) and 6 (10.9%), respectively, had pendrin autoantibodies.

• The prevalence of pendrin autoantibodies did not differ significantly among the ATD patient cohorts and the healthy control group (P=0.186 and P=0.317 for GD and AH patients, respectively) nor between the GD and AH patient groups (P=0.76).

2. Pendrin autoantibody titres

• Immunoreactivity against pendrin could be detected in 3/5 AH and 3/7 GD patient sera at a dilutions of up to 1:500 (as determined from a pendrin Ab index above the upper limit of normal for the RBA) (Fig 2a).

• For 2/3 AH and 2/7 GD patients, pendrin autoantibodies were detected in serum dilutions of up to 1:1000 (Fig 2a).

3. Pendrin autoantibody specificity

• Pre-absorption with increasing amounts of non-radio labelled pendrin reduced pendrin autoantibody binding of all 12 pendrin autoantibody-positive ATD patient sera in the RBA (Fig 2b): pendrin Ab indices of serum samples pre-absorbed with pendrin were significantly lower than those of unabsorbed sera (P values were <0.05).

• Pre-absorption of pendrin autoantibody-positive ATD patient sera with either thyroside or MOHR1 did not affect pendrin autoantibody binding in the RBA (data not shown).

Summary of Findings

• A novel radioligand binding assay for pendrin autoantibodies was designed.

• Pendrin autoantibodies were found occur at a low prevalence in patients with ATD.

Acknowledgment

We thank Professor Richard Trembath (Department of Medical and Molecular Genetics, King’s College London, School of Medicine, Guy’s Hospital, London, UK) for providing plasmid pcDNA3-PDS.