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Autoantibody against Thyroid Iodide Transporter in the Sera from Patients with Hashimoto's Thyroiditis Possesses Iodide Transport Inhibitory Activity

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Recently we have newly identified an autoantibody against thyroid iodide transporter (TIT) in the sera from patients with autoimmune thyroid disease. In order to study the function of these autoantibodies, we established CHO-K1 cells stably expressing recombinant rat TIT (CHO-TIT cells), and the effect of IgGs from the patients with Hashimoto's thyroiditis on iodide uptake activity of CHO-TIT cells was investigated. We found that 4 out of 34 sera from patients with Hashimoto's thyroiditis strongly recognized TIT by Western blot analysis. These 4 IgGs, purified by protein A column chromatography, caused 14 to 62% inhibition of I^- accumulation in CHO-TIT cells. Next, using synthetic peptides, we determined the recognition site of the autoantibody on the TIT molecule. The autoantibody reacted with the synthetic peptide corresponding to the 6th extracellular loop of the TIT molecule. These results suggest that autoantibody against TIT in the sera from patients with Hashimoto's thyroiditis binds to the 6th extracellular loop of TIT protein and inhibits I^- transport into the thyrocytes. Anti-TIT autoantibody might participate in the pathogenesis of Hashimoto's thyroiditis and modulate thyroid function of patients with the disease.

There are two major antigens in Hashimoto's thyroiditis, e.g thyroglobulin (Tg) and thyroid peroxidase (TPO). Autoantibody against Tg or TPO is useful for the diagnosis of the disease, but in many patients, high titer of anti-TPO or anti-Tg antibody is present for many years without development of hypothyroidism (1). So, the role of these autoantibodies in the pathophysiology of the disease has long been debated actively (2).

Recent cloning of TIT cDNA has revealed that it encodes intrinsic membrane protein with 12 putative transmembrane domains and that the gene is primarily expressed in the thyroid (3). Subsequently, using recombinant TIT protein, we newly identified autoantibody against TIT in the sera from patients with autoimmune thyroid disease (4). However, the function of autoantibody against TIT and its recognition site(s) remain to be elucidated. In the present study, we examined the effect of anti-TIT autoantibody on recombinant TIT activity and further analyzed its recognition site(s) on the molecule.

MATERIALS AND METHODS

Western blot analysis was performed using FRTL-5 cell membrane as described previously (4). Rabbits antibody against rat TIT was obtained by immunizing them with glutathione S-transferase/N-terminal portion of TIT fusion protein (4).

Rat TIT cDNA (−29 to 1975 bp, A in ATG is designated as +1) was cloned from FRTL-5 cells (4), and ligated into eukaryotic expression vector pSG_5 (pSG-TIT). The construct was then transfected into CHO-K1 cells by electroporation. Co-transfection with pRc/CMV was performed with resistance to G418 used as a selectable marker for transfection. As a control, pSG_5 with pRc/CMV was also transfected into CHO-K1 cells.

Diagnosis of Hashimoto's thyroiditis was made on the basis of elevated anti-TG or anti-TPO antibody titer in patients with a hard diffuse goiter without thyrotropin receptor antibody, and additional evidence by fine needle aspiration biopsy. Purification of IgGs from patients' sera was carried out using ImmunoPure IgG Purification Kit

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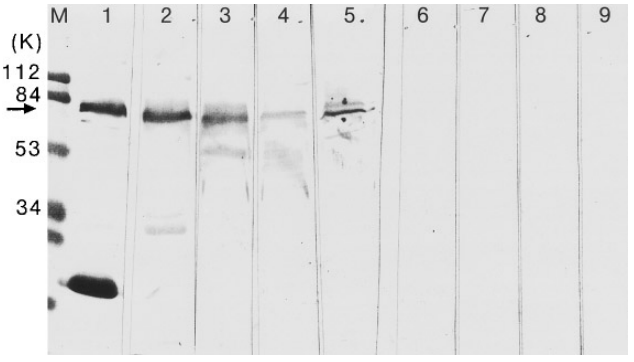

FIG. 1. Western blot analysis of TIT by sera of Hashimoto's thyroiditis. Membrane fraction from FRTL-5 cells (15 μg) was electrophoresed in 10% polyacrylamide-0.1% SDS and transferred to nitrocellulose filter. The filters were incubated with rabbit antiserum to TIT (1:500) (lane 1), Hashimoto's sera (1:500) from patients 1 to 4 (lanes 2 to 5) and sera (1:500) from normal subjects 1 to 4 (lanes 6 to 9), and then stained with peroxidase conjugated anti-human IgG (Jackson Immunoresearch, 1:1000). M: molecular weight marker.

(PIERCE), and before use, there were extensively dialyzed to Hanks' balanced solution. I^- uptake assay was performed in the presence of 100 nM ^{125}I (106 mCi/mmol) essentially by the methods of Weiss et al. (5).

Thirty peptides covering the full extracellular domain of rat TIT, each of which consists of 9 to 10 amino acids, were synthesized on cellulose sheets using SPOTs kit (Cambridge Research Biochemicals). Reactivity of patient's IgGs with each peptide was detected with β-galactosidase labeled anti-human IgG using 5-bromo-4-chloro-3-indoyl-β-D-galactopyanide as substrate.

RESULTS AND DISCUSSION

Using recombinant rat TIT protein blotted on nitrocellulose sheet, we screened 34 sera of Hashimoto's thyroiditis for their binding ability to the protein, and found that 4 of them recognized it (4). To confirm that these 4 IgGs truly react with TIT protein, we performed Western blot analysis using the membrane fraction of FRTL-5 cells (Fig. 1). All of these IgGs, especially those from patients 1 and 2, strongly stained 80 KDa protein, which co-migrated with the band recognized by rabbit antibody toward TIT (4).

We then studied the effect of these anti-TIT autoantibodies on I^- transport activity using CHO-K1 cells expressing recombinant TIT (CHO-TIT). Fig. 2a shows I^- uptake activity of CHO-TIT cells. I^- accumulation in the cells increased linearly up to 10 min, but no activity was observed in the CHO-K1 cells transfected with pSG_5. Fig. 2b shows the effect of anti-TIT positive IgGs from the patients on I^- transport activity of CHO-TIT cells. In the presence of patient's IgGs, CHO-TIT cells accumulated 38 to 86% of I^-, when the mean value of the cells in the presence of healthy subjects' IgG was accounted as 100%. It is particularly interesting that IgGs from patients 1 and 2, which strongly stained TIT by Western analysis, showed relatively potent inhibitory activity on I^- uptake by the cells.

We further analyzed the recognition site(s) of anti-TIT autoantibody on the molecule using synthetic peptides corresponding to the extracellular domain of the TIT. As shown in Fig. 3, the autoantibody from patient 1 reacted with peptide P23 (PPGEQTMGV, amino acid No.472 to 481) which corresponds to N-terminal site of 6th extracellular loop of the TIT molecule. The peptide was also recognized by serum from patient 2 (data not shown).

The sera of patients suffering from Hashimoto's thyroiditis contained heterogeneous autoantibodies such as anti-TPO antibody or anti-Tg antibody, and in some cases, anti-thyrotropin receptor antibody. It has been demonstrated that small portion of anti-TPO antibody inhibited the enzyme activity in vitro, although this remains controversial (6-9). In addition, some portion of anti-TPO antibody has also been reported to show antibody-dependent cytotoxicity (10).

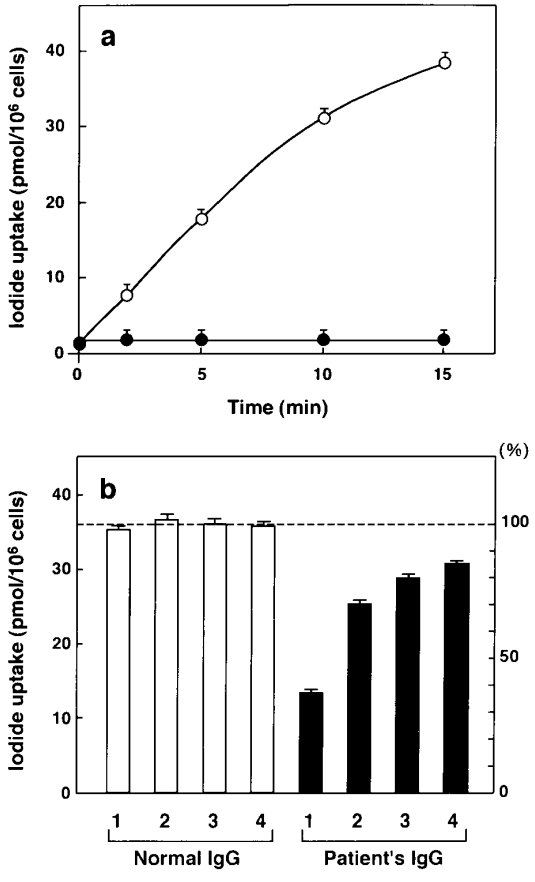

FIG. 2. (a) Iodide uptake activity of CHO-K1 cells stably expressing TIT. CHO-K1 cells were transfected with pSG-TIT (○ — ○) or pSG_5 (● — ●). Each point represents the of triplicate determinants. (b) Effects of purified IgGs from control subjects (open column) and patients with Hashimoto's thyroiditis (closed column) on iodide uptake activity of CHO-TIT cells. After preincubation of the cells with purified IgG (500 μg/ml) at 37°C for 1 h, 100 nM of ^{125}I was added, and further incubated for 10 min. Accumulated ^{125}I was determined by permeabilizing the cells with ethanol for 20 min. Each value represents the mean of triplicate determinants. The mean uptake level of the cells in the presence of normal IgGs was accounted as 100% and shown as a dotted line.

If anti-thyrotropin receptor antibodies (thyroid-stimulating antibody and/or TSH stimulation blocking antibody) exists, it is possible for them to stimulate or inhibit I^- transport into the thyrocytes through the cAMP mediated mechanism (11,12).

Therefore, at present, if we employ thyrocytes in primary culture or a thyroid epithelial cell line such as FRTL-5 for evaluating the function of anti-TIT autoantibody, it seems difficult to interpret the results. So, here, we used non-thyroid cells expressing recombinant TIT, but not TPO, Tg, thyrotropin receptor.

Preliminarily, we studied the effect of normal and patient's sera on I^- uptake activity of CHO-TIT cells. We observed that some patient's sera, which did not recognized TIT by Western blot analysis, as well as some normal sera, caused 90% or more inhibition of I^- uptake. However, after dialyzing to the buffer, these sera lost their inhibitory activity on I^- uptake by the cells (data not shown). This means that some sera contain dialysable inhibitor(s) to TIT activity and the use of purified IgG is necessary for the accurate evaluation of the autoantibody activity.

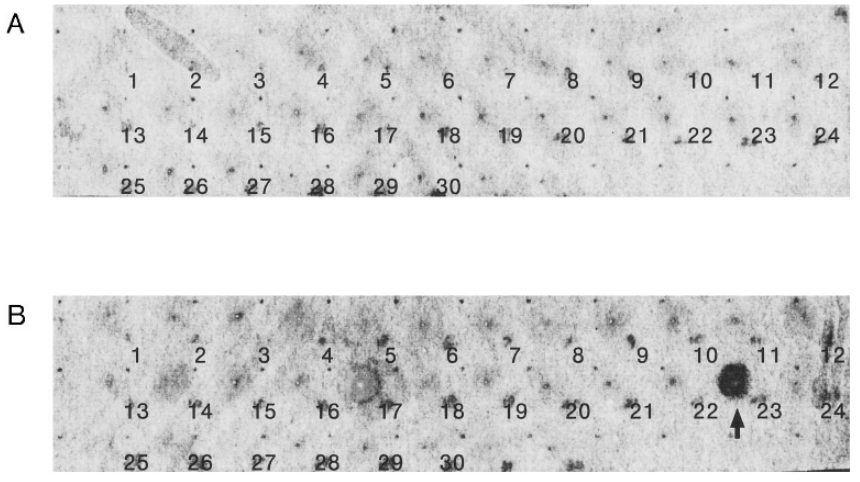

FIG. 3. Recognition site of anti-TIT autoantibody on the TIT molecule. Thirty peptides(P1 ~P30) corresponding to the extracellular loops of rat TIT were synthesized on cellulose sheets. Spot number is identical to that of peptide. Sheets A and B were incubated with serum (1:500) from normal subject 1 and patient 1, respectively. Positive signal was developed with β-galactosidase labeled anti-human IgG (1;1000). Arrow indicates a blue positive signal.

Using CHO-TIT cells and purified IgG, we obtained the clear results that high titer of anti-TIT antibody possesses I^- transport inhibitory activity. Although further elucidation is needed for true prevalence of the antibody, it seems likely that anti-TIT autoantibody might be involved in the pathogenesis of Hashimoto's thyroiditis and might modulate thyroid function of the patients with Hashimoto's thyroiditis.

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