Isolation of a Complementary DNA Clone for Thyroid Microsomal Antigen
Homology with the Gene for Thyroid Peroxidase

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Abstract
The thyroid microsomal antigen (MSA) in autoimmune thyroid disease is a protein of ~107 kD. We screened a human thyroid cDNA library constructed in the expression vector lambda gt11 with anti–107-kD monoclonal antibodies. Of five clones obtained, the recombinant β-galactosidase fusion protein from one clone (PM-5) was confirmed to react with the monoclonal antiserum. The complementary DNA (cDNA) insert from PM-5 (0.8 kb) was used as a probe on Northern blot analysis to estimate the size of the mRNA coding for the MSA. The 2.9-kb messenger RNA (mRNA) species observed was the same size as that coding for human thyroid peroxidase (TPO). The probe did not bind to human liver mRNA, indicating the thyroid-specific nature of the PM-5-related mRNA. The nucleotide sequence of PM-5 (842 bp) was determined and consisted of a single open reading frame. Comparison of the nucleotide sequence of PM-5 with that presently available for pig TPO indicates 84% homology. In conclusion, a cDNA clone representing part of the microsomal antigen has been isolated. Sequence homology with porcine TPO, as well as identity in the size of the mRNA species for both the microsomal antigen and TPO, indicate that the microsomal antigen is, at least in part, TPO.

Introduction
For more than two decades, antibodies against an unidentified thyroid microsomal antigen have been observed and studied in patients with autoimmune thyroid disease, in particular Hashimoto's thyroiditis (1, 2). In the past two years, rapid progress has been made in elucidating the nature of this microsomal antigen. By Western blot analysis (3) and by immunoprecipitation (4, 5), the microsomal antigen has been identified as a protein of ~107,000 D. Furthermore, there is strong evidence accumulating that the microsomal antigen is thyroid peroxidase (TPO). Thus, using sera from patients with Hashimoto's thyroiditis, a significant correlation was observed between microsomal antibody titers and anti-TPO activity (6, 7). TPO bioactivity can be immunoprecipitated by sera containing microsomal antibodies (6, 8). Finally, immobilized, purified TPO can selectively adsorb antimicrosomal, but not antithyroglobulin, antibodies (9).

Recently, monoclonal antibodies have been produced against the 107-kD human thyroid microsomal antigen (10). In the present report we describe the use of these monoclonal antibodies to obtain a complementary DNA (cDNA) clone representing part of this antigen. Comparison of the nucleotide sequence from part of this clone with the nucleotide sequence of porcine thyroid peroxidase (11, 12) now demonstrates the identity of these two antigens, at least in part.

Methods

Screening of human thyroid cDNA library. The construction of a human thyroid cDNA library in the expression vector lambda gt11 (13) has been previously described (14). This library consists of ~10^6 recombinants with an average insert size of 1.1 kb. The expanded library was plated (~3 x 10^6 plaque-forming U/150-mm dish in Escherichia coli Y1090 cells). After 3–3.5 h, nitrocellulose filters previously saturated with isopropyl β-D-thiogalactopyranoside were applied for 3 h. The filters were then exposed to antimicrosomal monoclonal antibodies (1:100 dilution) in asctic fluid according to the method of Huynh et al. (15) with the exception that bound antibody was detected with anti–mouse IgG (Cappel Laboratories, Cochranville, PA) conjugated to horseradish peroxidase (CooperBiomedical, Inc., Malvern, PA) (1:100 dilution overnight at 4°C). The peroxidase substrate was 0.5 mg/ml 4 chloro-1-naphthol (Sigma Chemical Co., St. Louis, MO), 10 mM imidazole, 0.0125% H2O2.

Western blot analysis. Lysogens of selected lambda gt11 clones were made in Y1089 cells for generation of fusion proteins (15). Bacterial lysates were prepared by freeze-thawing and sonication (15). Aliquots (~20 μg protein) were applied to 7.5% polyacrylamide gels. The electrophoresed proteins were transferred onto nitrocellulose paper as previously described (14). The filters were then rinsed once with Tris-buffered saline (TBS) buffer (50 mM Tris, pH 8.0, 150 mM NaCl) and then for 20 min in TBS containing 20% fetal calf serum, followed by the same protocol used to screen the library. As a control, mouse ascitic fluid IgG 1 monoclonal antiserum (Cappel Laboratories) was used.

Northern blot analysis. Poly(A)+ messenger RNA (mRNA) was prepared from Graves' thyroid tissue and from normal human liver tissue by the method of Chirgwin et al. (16), followed by oligo(dT) affinity chromatography. mRNA (7 μg/lane) was applied to 2.2 M formaldehyde agarose gels (1.5%) and transferred by blotting to Nytran paper (Schleicher & Schuell, Inc., Keene, NH). The paper was probed with the indicated cDNA inserts, recovered from pUC18 into which they had been subcloned from lambda gt11. The probes consisted of

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1. Abbreviation used in this paper: TPO, thyroid peroxidase.

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of Bionet. Comparison probes (14, 18). Y1089 was an interestingly, the PM-5 fusion protein, and the monoclonal antiserum to the transferred proteins shown in lane I. Lane J demonstrates lack of interaction of a control monoclonal antibody with the same proteins.

the entire PM-5 cDNA and a 0.6-kb pig TPO cDNA, fragment J (11). The probes were labeled by nick translation (17) to a specific activity of ~ 2 × 10⁶ cpm/µg. Hybridization conditions were as previously described (14, 18).

Nucleotide sequencing. cDNA was subcloned into Ml3mp18 and Ml3mp19 and sequenced by the dideoxy termination method (19). Comparison of nucleotide sequences was made by the I- FIND program of Bionet.

Results

Screening of the human thyroid cDNA library in lambda gt11 with monoclonal antibodies against the 107-kD human thyroid microsomal antigen yielded five clones. After plaque purification, the phage from each clone was used to make lysogens in Y1089 bacteria (15), and the bacterial lysates from these were used for Western blot analysis. The recombinant β-galactosidase fusion protein in only one clone (PM-5) was confirmed to react with the monoclonal antiserum (Fig. 1). Interestingly, this fusion protein was a doublet. Control monoclonal antisera of the same subtype did not react with this fusion protein (Fig. 1).

The cDNA insert from PM-5 (~ 0.8 kb) was isolated, subcloned into the Eco RI site of pUC18, and subsequently used as a probe to estimate the size of the mRNA coding for the entire protein. Northern blot analysis indicated hybridization to a 2.9-kb species (Fig. 2). The probe did not bind to human liver mRNA, indicating the thyroid-specific nature of the PM-5-related mRNA. When a 0.6-kb cDNA probe from porcine TPO was hybridized to human thyroid mRNA, a similar 2.9-kb mRNA species was identified (Fig. 2). As with PM-5, the TPO probe did not bind to human liver mRNA.

Because of the previous evidence that the microsomal antigen is TPO, and because of the identical sizes of mRNA

Figure 1. Western blot analysis of the PM-5 fusion protein. The fusion protein of PM-5 was generated in a Y1089 lysogen. Aliquots of bacterial lysate (20 µg protein) were applied to a 7.5% polyacrylamide gel. After electrophoresis and transfer to nitrocellulose paper, the proteins were probed with antimicrosomal antigen monoclonal antibody (see Methods). Lane I shows Coomassie Blue staining of the gel. Molecular weight markers were myosin (205 kD), β-galactosidase (116 kD), rabbit muscle phosphorylase B (97 kD), bovine serum albumin (66 kD), and egg albumin (45 kD). Lane 2 indicates binding of specific monoclonal antiserum to the transferred proteins shown in lane I. Lane J demonstrates lack of interaction of a control monoclonal antibody with the same proteins.

Figure 2. Northern blot analysis of human thyroid and liver mRNA. 7 µg each of human thyroid mRNA (lane T) and human liver mRNA (lane L) were electrophoresed on a 1.5% agarose gel and were then transferred to Nytran paper as described in Methods. The mRNA was probed with the nick-translated 0.8-kb cDNA insert from clone PM-5 (left panel) and with a 0.6-kb porcine TPO cDNA fragment J (right panel) (11). Sizing of the 2.9-kb mRNA species was on the basis of DNA and RNA ladders (Bethesda Research Laboratories, Gaithersburg, MD).

Figure 3. Nucleotide and amino acid sequence of clone PM-5. The translational reading frame begins at basepair No. 2 in the PM-2 sequence.
species for the human microsomal antigen, human TPO (Fig. 2) and pig TPO (11), we determined the entire nucleotide sequence of PM-5. The human thyroid microsomal antigen cDNA clone (PM-5) consisted of a tract of 842 bp, all within a single reading frame (Fig. 3). Remarkably, comparison of the nucleotide sequence of PM-5 with that of pig TPO revealed an homology of 84% (Fig. 4). This region of homology is located between 2.2 kb and 1.4 kb upstream from the 3' end of the pig TPO cDNA gene (12).

Discussion

Pig TPO was recently cloned in our laboratory using synthetic oligonucleotide probes based on the amino acid sequence of tryptic fragments of highly purified porcine TPO (11, 12). In the present study we screened a human cDNA lambda gt 11 library by another method, namely using monoclonal antibodies to detect an expressed protein. Clones with regions of homologous sequence were obtained by each procedure. The < 100% homology between the regions of the two clones is to be expected because they are from two different species, namely pig and human. Despite the fact that the entire gene has not been characterized, the degree of homology noted, as well as the identity in the sizes of mRNA for both the microsomal antigen and TPO, indicate that the microsomal antigen is, at least in part, TPO.

It is interesting that the fusion protein generated by clone PM-5 is a doublet. Because PM-5 is clonal it follows that the fusion protein generated by this clone is derived from one gene, and therefore the doublet is likely to be the result of posttranslational modification of the fusion protein. However we cannot exclude the possibility of multiple transcriptional initiation sites, although this is highly unlikely in view of the fact that the cDNA insert is within the β-galactosidase gene, and transcriptional initiation will begin in the β-galactosidase gene itself.

The present data indicating identity of the thyroid microsomal antigen and TPO genes open the way for the complete characterization of these antigens. This in turn will be very useful in further studies on the pathogenesis of autoimmune thyroid disease. For example, it will permit the development of (a) more sensitive and specific assays for autoantibodies, (b) the generation of antibodies against specific epitopes on the antigen that can be used for in vivo and in vitro studies on the pathogenesis of autoimmune thyroid disease, (c) potential therapy for Hashimoto’s thyroiditis using antidiotypic antibodies, and (d) there is now the opportunity for examining the mechanism of regulation of microsomal antigen expression in autoimmune thyroid disease, as well as the relationship between the expression of this gene and other genes such as for the class II histocompatibility antigens.

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Note added in proof. Since submitting the present paper we have determined the complete nucleotide sequence of both pig and human TPO cDNA. They are both 3.2 kb in length. Irrespective of whether a

Figure 4. Homology of the nucleotide sequences of clone PM-5 (human) and pig TPO (pTPO). Colons indicate nucleotide identity. The numbering of the pig TPO nucleotide sequence is arbitrary because we have not as yet determined the exact length of the gene extending up to the 5' end. However this region of pig TPO spans from 2.2 kb to 1.4 kb from the 3' end (12).
pig or human TPO cDNA probe was used, the same size mRNA species was detected (as shown in Fig. 2).

References


