Vol. 179, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
August 30, 1991 Pages 372-377

A HUMAN FAB FRAGMENT SPECIFIC FOR THYROID PEROXIDASE GENERATED BY CLONING THYROID LYMPHOCYTE-DERIVED IMMUNOGLOBULIN GENES IN A BACTERIOPHAGE LAMBDA LIBRARY

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Received June 28, 1991

SUMMARY A human Fab fragment (SP2) which binds specifically to human thyroid peroxidase has been generated by expressing random combinations of heavy and light chain immunoglobulin genes (derived from Graves' thyroid cDNA) in a bacteriophage lambda library. In common with many serum TPO autoantibodies, the cloned Fab fragment is IgG1 kappa and has a high affinity for TPO ($\sim 10^{-9}$ M). On the basis of their nucleotide sequences, the heavy and light chain genes coding for SP2 belong to families VHI,(D),JH3 and VKI,JK2, respectively. These data provide the first characterization at a molecular level of a human thyroid peroxidase antibody associated with autoimmune thyroid disease.

IgG autoantibodies to thyroid peroxidase (TPO) are characteristic of patients with Graves' and Hashimoto's diseases and are implicated in autoimmune thyroid destruction (reviewed in 1). The availability of monoclonal human TPO autoantibodies of the same class and high affinity as those present in serum would contribute significantly towards an understanding of the pathogenesis of these common autoimmune diseases. Unfortunately, despite numerous attempts using EB virus infection and/or cell fusion, only one cell line secreting IgG class human autoantibody to TPO has been produced, and this human-mouse hybridoma was unstable (2).

Recently a technique has been described for cloning heavy and light chain gene fragments in a bacteriophage expression library (3). We now report the use of this approach to clone a human Fab fragment specific for human TPO.

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Abbreviations : TPO : thyroid peroxidase; H chain : immunoglobulin heavy chain; L chain : immunoglobulin light chain; VH region : variable region of the heavy chain; VL : variable region of the light chain.

0006-291X/91 $1.50

MATERIALS AND METHODS

Construction of combinatorial H and L gene libraries: A cDNA library from the thyroid gland of a patient with Graves' disease (4) was used as a source of cDNA coding for thyroid autoantibodies. The presence of thyroid autoantibody mRNA in this gland was previously suspected for two reasons. First, infiltrating thyroid lymphocytes are a major source of thyroid autoantibodies (5,6). Second, proteins expressed by this library were recognized by antiserum to human IgG (unpublished observations). A combinatorial library of heavy (H) chain fragments and kappa light chain genes was produced using oligonucleotides and vector in the Immunozap Cloning Kit (Stratacyte, La Jolla, CA). Bacteriophage DNA prepared (7) from the Graves' thyroid cDNA library was used as template in the polymerase chain reaction (8). Heavy chain gene sequences were amplified in separate reactions using 4 different forward oligonucleotide primers corresponding to the relatively conserved amino terminus of the molecule and a reverse primer to the CH1 domain-hinge junction of IgG1. Kappa light chain genes were amplified using primers complementary to the sequence coding for the signal peptide/kappa light (L) chain junction and the carboxyl terminus. The combinatorial library was constructed according to the protocol of Stratacyte.

Screening for TPO binding : The unamplified combinatorial library was screened in XL1-Blue cells by conventional techniques (7) using highly-purified, secreted recombinant human TPO (9) labelled with ^{125}I by the iodogen method (10) to a specific activity of 10 uCi/ug. A plaque expressing a Fab fragment that bound radiolabeled TPO was identified by autoradiography and was cloned to homogeneity. The affinity of this TPO antibody was measured in a confluent plaque lysis assay (~100 plaques per spot) by the addition of unlabeled TPO (10^{-10} M - 10^{-6} M) to the ^{125}I-labeled TPO. Densitometry was performed (Biorad 620 video-densitometer) on duplicate spots at each TPO concentration and expressed as arbitrary OD (optical density) units.

Nucleic acid sequencing of H and L genes coding for human TPO specific Fab fragment : The nucleotide sequence of the cDNA of the TPO-positive clone (SP2) was determined (11) in both directions following its recovery in Bluescript using the helper phage R408 (Stratacyte).

RESULTS

Screening ~2 x 10^5 plaques yielded one (SP2) that bound radiolabeled TPO. After cloning to homogeneity, the affinity for TPO of the Fab fragment expressed by SP2 was determined by competition studies with unlabeled recombinant TPO and was found to be ~10^{-9} M (Fig. 1). The specificity of this interaction was evident by the inability of the Fab fragment to bind radiolabeled thyroglobulin, another major thyroid autoantigen (data not shown).

Comparison of the nucleotide sequence of the IgG heavy (H) chain (Fig. 2) and light (L) chain (Fig. 3) with known germline sequences characterizes this TPO binding Fab fragment. Specifically, the VH gene belongs to the VHI family with 91.2% homology to the 1-1 germline gene (12). The D segment contains three of the 5 nucleotide motifs shared by D_M, D_N and D_{LR} (GGTAT) and D_{LR} (TACTA, GTATG) (12). Because of very low homology with reported D region nucleotide sequences, it is difficult to assign the D region of SP2 to a particular gene family. The J segment is a JH3 which appears to be truncated at its 5' end (12).

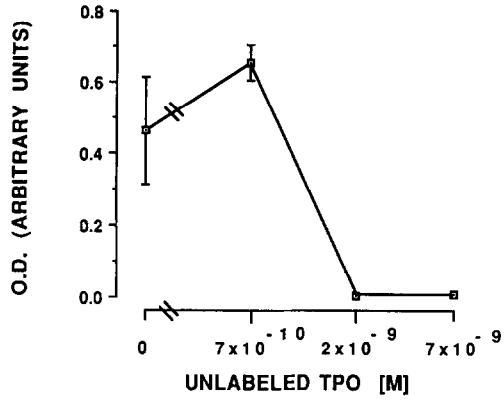

Fig. 1. Binding affinity of Fab fragment SP2 for recombinant human thyroid peroxidase (TPO). Brackets indicate the mean ± the range of duplicate densitometric values obtained for each TPO concentration in a representative experiment. Comparable results were obtained in two additional experiments.

VH SEGMENT (VH1)

```
       Q  V  K  L  L  E  S  G  A  E  V  K  K  P  G  A  S  V  K  V
SP2    CAGGTGAAACTGCTCGAGTCTGGGGCTCAGCTCAAGAAGCCTGCGGCCTCAGTGAAGGTC    60
1-1    ------CAG---G-GC-----C----------------T-------------------

                                        CDR1
                                      ***************
       S  C  K  A  S  G  Y  T  F  T  G  H  Y  M  H  W  V  R  Q  A
SP2    TCCTGCAAGGCTTCTGGATACACCTTCACCGGCCACTATATGCACTGGGTGCGACAGGCC   120
1-1    --------------------------------T-------------------------

                                              CDR2
                                  ****************************************
       P  G  Q  G  L  E  W  I  G  W  I  S  P  N  R  G  A  T  R  F
SP2    CCTGGACAAGGGCTTGAGTGGATAGGATGGATCAGCCCTAACAGAGGTGCCACAAGGTTT   180
1-1    ----------------------G----------A---------T----G---C-AC-A-

       ******************
       A  Q  K  F  Q  G  R  V  T  M  T  S  D  T  S  I  N  T  V  Y
SP2    GCACAGAAGTTTCAGGGCAGGGTCACCATGACCAGCGACACGTCCATTAACACAGTCTAC   240
1-1    ----------------------------------G-----------C-G-----C----

       M  E  L  S  G  L  R  F  D  D  T  A  V  Y  Y  C  A  T
SP2    ATGGAGCTGAGCGGCCTGAGATTTGACGACACGGCCGTGTATTACTGTGCGACA
1-1    ------------AAG-------C--------------------------G-
```

D SEGMENT

```
              CDR3
       ***************************
       T  R  T  A  Y  Y  G  M  D
SP2    ACACGCACGGGCTACTACGGTATGGAC
```

JH SEGMENT (JH3)

```
                 R  L  G  P  R  D  H  G  H  R  L  F
SP2    ..........GTCTGGGGCCAAGGGACCACGGTCACCGTCTCTTCA
JH3    ATGCTTTTGAT----------------A-T---------------
```

Fig. 2. Nucleotide and derived amino acid sequence of the SP2 heavy chain compared with the VH1 germline gene 1-1 and the JH3 germline gene (12). The complementarity determining regions (CDR) are indicated by the asterisks. Identical nucleotides are depicted by dashes and deleted nucleotides by dots.

VL SEGMENT (VKI)

```
       E  L  V  M  T  Q  S  P  S  S  L  S  A  S  E  G  D  T  V  T
SP2    GAGCTCGTGATGACCCAGTCTCCATCTTCCCTGTCTGCATCTGAGGGAGACACAGTCACC    60
KLVJ   --CA--CA-----------------C---------------TA-------G-------

                          CDR1
                 *********************************
       I  T  C  R  A  S  E  N  I  S  R  Y  S  N  W  Y  Q  Q  Q  P
SP2    ATCACTTGCCGGGCAAGTGAGAATATTAGCAGGTATTCAAATTGGTATCAGCAGCAACCA   120
KLVJ   -----------------C---GC-----T-AC----T---------------A-----

                                    CDR2
                                 **********************
       G  K  A  P  K  L  L  I  S  A  A  S  T  L  Q  S  G  V  P  S
SP2    GGGAAAGCCCCTAAACTCCTGATCTCTGCTGCATCCACTTTACAAAGTGGGGTCCCATCA   180
KLVJ   -------------G----------A-----------G---G------------------


       R  F  S  G  S  G  S  G  T  H  F  T  L  T  I  N  S  L  Q  P
SP2    AGGTTCAGTGGCAGTGGATCTGGGACACATTTCACTCTCACCATCAACAGTCTGCAACCT   240
KLVJ   --------------------------G-----------------G-------------

                                  CDR3
                               ************************
       G  D  F  A  T  Y  Y  C  Q  Q  T  Y  S  S  P  F
SP2    GGAGATTTTGCAACTTACTACTGTCAACAGACTTACAGTTCCCCGTTC
KLVJ   -A----------------------------G-------A---ACC-T
```

J SEGMENT (JK2)

```
       T  F  G  Q  G  T  K  L  E  I  K  R  T
SP2    ACTTTTGGCCAGGGGACCAAGCTGGAGATCAAACGAACT
KV312  ---------------------------------------
```

Fig. 3. Nucleotide and derived amino acid sequence of the SP2 light chain compared with the VKI germline gene HUMIGKLVJ (GenBank accession number D90158) and the JK2 germline gene in Taykv312 (13). The complementarity determining regions (CDR) are indicated by the asterisks. Identical nucleotides are depicted by dashes.

The light chain is coded for by a VKI which is 89.6% homologous with the germline gene HUMIGKLVJ (GenBank accession number D90158). The light chain J segment is a JK2 (13).

DISCUSSION

The expression in bacteria of random combinations of heavy and light chain immunoglobulin cDNA genes has previously been used to generate human Fab fragments which bind tetanus toxoid using cDNA from individuals immunized with this antigen (14,15). However, there are no previous reports on the production of disease-associated human autoantibodies using this system. In the present study, we have generated a human Fab fragment which binds a major thyroid

autoantigen, TPO. The cDNA used for this purpose was transcribed from mRNA prepared from Graves' thyroid tissue which is enriched in B-lymphocytes capable of producing thyroid autoantibodies (5,6).

Antibodies to TPO in patients with autoimmune thyroid disease are frequently of subclass IgG1 and/or IgG4, with kappa light chains predominating (16). For this reason our initial approach was to construct and screen an IgG1-kappa combinatorial cDNA library. On the basis of available information, the VH and VL genes of SP2 appear to be moderately mutated forms of germline gene families VHI and VKI. The D region, which does not resemble any reported germline sequence, is likely to contribute further to recognition of TPO by SP2. It is not known whether this particular heavy and light chain combination reflects the in vivo situation. However, the high affinity of SP2 was comparable with the affinities of TPO autoantibodies present in patients with autoimmune thyroid disease (1.1×10^{-9} M - 9.4×10^{-8} M)(17). Therefore, our data are likely to provide the first characterization at a molecular level of a human thyroid peroxidase antibody associated with autoimmune thyroid disease.

ACKNOWLEDGMENTS

This research was supported by NIH grants DK36182 and EY09498. We thank Ms. Carmela Filamor for expert administrative assistance and Dr. Scott Hutchinson of the Nichols Institute, San Juan Capistrano, CA, for providing us with purified recombinant human TPO.

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