

Molecular characterization of the V regions of four IgE antibodies specific for trichosanthin

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SUMMARY

We report here the cDNA cloning and variable region nucleic acid sequences of the heavy and the light chains of four monoclonal IgE and one monoclonal IgG1 antibodies against trichosanthin (TCS), all of which recognized a similar or related antigenic determinant. Sequence analysis revealed that the V_H regions of the four IgE antibodies use different V gene segments from the 7183, 36–60 and J558 families. The D_H genes varied extensively in nucleotide sequence and length, with D segments DSP2, DFL16 and DQ52 being used with equal frequency. All J_H gene segments except J_H1 were present in the ϵ transcripts. In contrast, there appeared to be restricted L-chain utilization. All four L-chain V genes cloned belonged to the V κ 21 family and a bias in the use of J κ 1 gene was also observed. The V_L sequences of the anti-TCS monoclonal antibodies showed much less diversity than the V_H sequences. We propose that the L-chain rather than H-chain may play a more significant role in specific binding with allergenic determinant on TCS.

INTRODUCTION

Trichosanthin (TCS), a plant protein of 27 000 MW, is an active component of a traditional Chinese medicine used for inducing abortion.^{1,2} Recently it has been found that TCS is able to inhibit tumour growth and human immunodeficiency virus (HIV) replication.³ Furthermore, it was shown to be a type-1 ribosome-inactivating protein with N-glycosidase activity.⁴ The primary amino acid sequence, nucleotide sequence, and the crystal structure of this molecule have all been worked out.^{5,6} However, TCS occasionally induces anaphylaxis in some patients, which limits its practical applications. To better understand the anaphylactic mechanism, we undertook a study of the TCS allergenic determinants. To do this, we induced TCS-specific IgE responses in mice, and raised 16 monoclonal antibodies (mAb) to TCS.⁷ Five of them were of the IgE class. Through competitive antibody binding enzyme-linked immunosorbent assays (ELISA), the 16 mAb could be classified into four groups.⁸ All five IgE mAb and two IgG1 mAb were in one group, which suggested that they might bind to similar or related sites on the TCS molecule. A rat anti-idiotypic mAb raised against one of the IgE mAb, TE1, reacted with two additional members of the same group.^{9,10}

Despite extensive research concerning the regulatory mechanism involved in specific IgE formation, only a few studies have dealt with the specific V-gene usage in IgE antibodies.^{11–13} We report here the cDNA cloning and sequence analysis of the variable region genes of four IgE and one IgG1 antibodies from the same group. The other IgE antibody, 4G8, was found to share

identical sequences with the IgE antibody TE2 in both V_H and V_L genes.

This research provides some information about the V region of allergic antibodies, and also provides a basis for further study of the relationship between the antigen combining site of the IgE and allergenic determinant of TCS.

MATERIALS AND METHODS

Hybridoma cell lines

Four hybridoma cell lines, TE1, TE2, 1G4 (TE3), 4C8 (TE4), secreting TCS-specific IgE, and one hybridoma cell line, 2A1, secreting TCS-specific IgG1, were all established in our laboratory. They all contain κ light chains. The hybridomas were generated by fusion of spleen cells from TCS/alum-immunized C57BL/6 mice with mouse NS-1 myeloma cells. A rat anti-idiotypic antibody, A1d6C5, was secreted by a rat–mouse hybridoma that was raised through fusion of TE1-immunized rat spleen cells with mouse NS-1 myeloma cells. It reacted significantly with only three of the anti-TCS mAb, TE1, 4C8 and 2A1. It reacted poorly with other anti-TCS mAb and there was no reaction against anti-DNP IgE mAb 142 and ADNP (kindly provided by Dr Metzger, NIH, Bethesda, MD) as well as normal mouse serum immunoglobulin.

Oligonucleotide primer design

Two pairs of degenerate primers common to V_H or V_L were synthesized according to the universal primers for murine V genes designed by Orlandi *et al.*,¹⁴ with some modifications (Table 1).

The J_H-FOR primer can be used interchangeably in different subclasses of heavy chains.¹⁴ In order to guarantee that the cloned V_H genes were definitely derived from ϵ heavy chains, a C ϵ -FOR primer was designed. It corresponds to the position of nucleotides

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(a)	10	(b)	10
VH61-1P	lys leu val gln pro gly gly ser arg GGC TTA GTG CAG CCT GGA GGG TCC CGG	VH207	asp val his leu gln glu ser gly pro asp leu val lys pro ser gln ser leu GAT GTG CAC CTT CAG GAG TCA GGA CCT GAC CTG GTG AAA CCT TCT CAG TCA CTT
TE1VH	Vh primer..... gln val gln leu gln glu ser gly gly CAG GTG CAG CTG CAG GAG TCA GGG GGA	4C8VH 2A1VH	glu lys --G --A-G --G -- --G --A-G --G --
VH61-1P	20 lys leu ser cys ala ala ser gly phe thr phe ser ser phe gly met his trp AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT AGT AGC TTT GGA ATG CAC TGG	VH207	20 ser leu thr cys thr val thr gly tyr ser ile thr ser gly tyr ser trp his TCA CTC ACC TGC ACT GTC ACT GGC TAC TCC ATC ACC AGT GGT TAT AGC TGG CAC
TE1VH	*.....CDR 1.....* val arg gln ala pro glu lys gly leu glu trp val ala tyr ile ser ser gly GTT CGT CAG GCT CCA GAG AAG GGG CTG GAG TGG GCA TAC ATT AGT AGT GGC	4C8VH 2A1VH	*..... trp ile arg gln phe pro gly asn lys leu glu trp met gly tyr ile his tyr TGG ATC CGG CAG TTT CCA GGA AAC AAA CTG GAA TGG ATG GGC TAC ATA CAC TAC
VH61-1P	40 ser ser thr ile tyr tyr ala asp thr val lys gly arg phe thr ile ser arg AGT AGT ACC ATC TAC TAT GCA GAC ACA GTG AAG GGC CGA TTC ACC ATC TCC AGA	VH207	40 trp ile arg gln phe pro gly asn lys leu glu trp met gly tyr ile his tyr TGG ATC CGG CAG TTT CCA GGA AAC AAA CTG GAA TGG ATG GGC TAC ATA CAC TAC
TE1VH	CDR 2.....* asp asn pro lys asn thr leu phe leu gln met thr ser leu arg ser glu asp GAC AAT CCC AAG AAC ACC CTG TTC CTG CAA ATG ACC AGT CTA AGG TCT GAG GAC	4C8VH 2A1VH	*..... ser asp ser ser asn tyr asn pro ser leu lys ser arg ile ser ile thr arg AGT GAT AGC TCT AAC TAC AAC CCA TCT CTC AAA AGT CGA ATC TCT ATC ACT CGA
VH61-1P	80 thr ala met tyr tyr cys ala arg ACG GCC ATG TAT TAC TGT GCA AGA	VH207	80 asp thr ser lys asn gln phe phe leu gln leu asn ser val thr thr glu asp GAC ACA TCC AAG AAC CAG TTC TTC CTG CAG TTG AAT TCT GTG ACT ACT GAG GAC
TE1VH	90 ser tyr tyr ser tyr phe ser tyr phe ser tyr glu gly --G TCC TAC TAT AGT TAC TTT AGT TAC GAG GGG	4C8VH 2A1VH	90 thr ala thr tyr tyr cys ACA GCC ACA TAT TAC TGT
JH4	*..... ala met asp tyr trp gly gln gly thr ser val thr val ser ser GCT ATG GAC TAC TGG GGT CAA GGA ACC TCA GTG ACC GTC TCC TCA	VH207	*.....CDR 3..... thr phe ala trp phe ala
TE1VH	*..... val arg ser asn ser val ala GCA AGA AGT AAC TCC GTG GCC	4C8VH	*..... ala arg ser asn ser val ala GCA AGA AGT AAC TCC GTG GCC
		2A1VH	*..... gly ala tyr asp gly ser GGC GCT TAC TAC GAT GGT AGC
		JH3	...* tyr trp gly gln gly thr leu val thr val ser ala TAC TGG GGC CAA GGG ACT CTG GTC ACT GTC TCT GCA
		4C8VH	Jh primer..... thr
		2A1VH	 thr --C AC--C T--

(c)	TE2VH	10 Vh primer..... gln val lys leu gln gly ser gly gly leu val lys pro gly gly ser leu CAG GTC AAG CTG CAG GGG TCA GGG GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG	
	VH98-3G	20 ala ser gly phe thr phe ser ser tyr ala met ser trp CA GCC TCT GGA TTC ACT TTC AGT AGC TAT GCC ATG TCT TGG	
	TE2VH	30 lys leu ser cys ala AAA CTC TCC TGT G--	*.....CDR 1.....*
	VH98-3G	40 val arg gln ser pro glu lys arg leu glu trp val ala glu ile ser ser gly GTT CGC CAG TCT CCA CAG AAG AGG CTG GAG TGG GTC GCA GAA ATT AGT AGT GGT	50 52a *.....
	TE2VH	60 gly ser tyr thr tyr pro asp thr val thr gly arg phe thr ile ser arg GGT AGT TAC ACC TAC TAT CCA CAG ACT GTG ACG GGC CGA TTC ACC ATC TCC AGA	70 *.....CDR 2.....*
(d)	VH98-3G	80 82a 82b 82c asp asn ala lys asn thr leu tyr leu glu met ser leu arg ser glu asp GAC AAT GCC AAG AAC ACC CTG TAC CTG GAA ATG AGC AGT CTG AGG TCT GAG GAC	
	TE2VH	90 thr ala met tyr tyr cys ala ACG GCC ATG TAT TAC TGT GCA AG	*.....CDR 3.....* --> JH3
	VH98-3G	D segment trp phe ala tyr trp gly gln TGG TTT GCT TAC TGG GGC CAA	
	TE2VH	arg asp arg ser arg --G GAC CGC -C-	
	JH3 TE2VH	gln thr leu val thr val ser ala GGG ACT CTG GTC ACT GTC TCT GCA	
(d)	VH205.12	10 gln val gln leu gln gln ser gly pro glu leu val lys pro gly ala ser val GAG GTC CAG CTG CAA CAA TCT GGA CCT GTG AAG CCT GGG GCT TCA GTG	
	1G4VH	20 Vh primer..... -----G--G-----	
	VH205.12	30 lys ile ser cys lys ala ser gly tyr thr phe thr asp tyr tyr met asn trp AAG ATA TCC TGT AAG GCT TCT GGA TAC ACG TTC ACT GAC TAC ATG AAC TGG	*.....CDR 1.....*
	1G4VH	40 val lys gln ser his gly lys ser leu glu trp ile gly asp ile asn pro asn GTG AAG CAG AGC CAT GGA AAG AGC CTT GAG TGG ATT GGA GAT ATT AAT CCT AAC	50 52a *.....
	1G4VH	60 asn -----A-----	
(d)	VH205.12	70 asn gly gly thr ser tyr asn gln lys phe lys gly lys ala thr leu thr val AAT GGT GGT ACT AGC TAC AAC CAG AAG TTC AAG GGC AAG GCC ACA TTG ACT GTA	
	1G4VH	80 tyr asp ser T--A--A--	
	VH205.12	90 asp lys ser ser ser thr ala tyr met glu leu arg ser leu thr ser glu asp GAC AAG TCC TCC AGC ACA GCC TAC ATG CAG CTC CGC AGC CTG ACA TCT GAG GAC	82a 82b 82c phe
	1G4VH	100 ser T--T--T--	
	JH2 1G4VH	gln gly thr thr leu thr val ser ser CAA GGC ACC ACT CTC ACA GTC TCC TCA	

Figure 1. (Pages 317 and 318.) Nucleotide and deduced amino acid sequences of the V_H of the five anti-TCS mAb and of their germ-line counterparts. (a) TE1, (b) 4C8 and 2A1, (c) TE2, (d) 1G4. Dashes signify identical nucleotides. Amino acid numbering and CDR are according to Kabat *et al.*¹⁷ The boundaries of the CDR are indicated (*). The positions of primers V_H -BACK and J_H -FOR are indicated. Sequences of germ-line genes: V_H 61-1P,¹⁸ V_H 207,^{19,20} V_H 98-3G,¹⁸ V_H 205-12,²¹ D and J_H sequences.^{17,22,23}

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Table 1. Primers for V region genes of heavy and light chains

Primers for V _H	
V _H -BACK: 5'-AGGT(G/C)(A/C)A(A/G) <u>CTGCAG</u> (C/G)AGTC(T/A)GG-3'	<u>PstI</u>
J _H -FOR: 5'-TGAGGAGACGGTGACCGTGGTCCCTTGGCCCCAG-3'	<u>BstEII</u>
Primers for V _L	
V _K -BACK: 5'-GACATTGAGCTCACCCAGTCTCCA-3'	<u>SacI</u>
J _K -FOR: 5'-GTTTGATCTCGAGCTTGGTGCC-3'	<u>XhoI</u>

32–53 in the C ϵ 1 region. C ϵ -FOR: 5'-GAAGCAGTGCCTTTA-CAGGGCT-3'.

Reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was prepared using guanidine hydrochloride–phenol–chloroform according to Chomczynski & Sacchi.¹⁵ The reverse transcription reaction was performed using 10 µg of the extracted total RNA as template; the reaction mixtures were denatured at 65° for 5 min, and gradually cooled down to room temperature. One microlitre (200 U) of Moloney murine leukaemia virus (MMLV) reverse transcriptase (BRL, Grand Island, NY) was added, followed by incubation at 37° for 1 hr. The first-strand cDNA was precipitated with ethanol at –20° for 2 hr; 1/5 of it was then taken as a template in PCR. The total reaction volume for PCR was 50 µl, including 4 µl of cDNA template, 5 µl of 10 × buffer for Taq DNA polymerase (Promega, Madison, WI), 2 µl 5 mM dNTPs, 25 pmole primer FOR, 25 pmole primer BACK, and 36 µl of water. After covering the surface with paraffin oil, the mixture was subjected to 94° to denature for 5 min, then 1 µl (3 U) Taq DNA polymerase (Promega) was added, followed by 35 cycles of denaturation for 1 min at 94°, annealing for 1.5 min at 50° and extension for 1.5 min at 72°. In the last cycle the extension was prolonged by 10 min. After completion of the PCR the overlaid paraffin was extracted with chloroform and the PCR products were precipitated with ethanol.

Gene cloning

The ends of heavy chain cDNA PCR products were blunted with Klenow DNA polymerase (BRL) at room temperature for 30 min. After being purified by electrophoresis in a 2% low melting-point agarose gel, they were ligated into a pBluescript/SK vector, which was previously digested with *SmaI* (Sino-American Biotech Co., Shanghai, China). The light chain cDNA PCR products were treated with two restriction enzymes, *SacI* (12 U/µl; Promega) and *XhoI* (12 U/µl; Promega), purified by electrophoresis in 2% low melting-point agarose gel, then ligated into pBluescript/SK vector, which was previously digested with *SacI* and *XhoI*. The ligation product was used to transform the competent host bacteria XL1-Blue, prepared by the CaCl₂ method according to Sambrook *et al.*¹⁶

Recombinant clones were selected and the presence of the inserted sequences was verified by PCR.

Sequence determination

Plasmid DNA was extracted by alkaline method, and the sequence was determined by the dideoxy (Sanger) sequencing method, using a TaqTrack Sequencing Kit (Promega). For each gene two to three independent clones were analysed until consistent sequences were obtained. The complementary determining region (CDR) and framework region (FR) in deduced amino acid sequences were numbered and defined according to Kabat *et al.*¹⁷

RESULTS

V genes of five anti-TCS mAb

The nucleotide sequences and the deduced amino acid sequences of the V_H and V_L of the five anti-TCS mAb are listed in Fig. 1 and Fig. 2. For V_L sequences, only four of the five mAb are shown because the V_L gene of 1G4 was not successfully cloned. The sequences are compared with their germ-line counterparts, and are numbered and the CDR defined (Figs 1 and 2). The BLAST search program was used to find nearly identical sequences in a non-redundant GenBank (National Center for Biotechnology, Bethesda, MD)/EMBL (European Bioinformatics Institute, Cambridge, UK)/DDBJ (Center for Information Biology, National Institute of Genetics, Mishima, Japan)/PDB (Brookhaven National Laboratory, Upton, NY) database; individual germ-line genes were identified by comparing with related germ-line V region sequences. The nucleotide sequence of V_K21D is not available in any database to the best of our knowledge. Therefore only the amino acid sequence of this germ-line V_K gene is shown in Fig. 2b.

V gene usage and somatic hypermutation of five mAb

In Table 2, the V gene family of V_H and V_L and the putative germ-line genes used by V, D and J gene segments of the heavy and light chains are listed. Among the five V_H sequences, V gene segments from three families were used: TE1 and TE2 used gene segments of V_H61–1P and V_H98–3G from the 7183 family; 4C8 and 2A1 used

Figure 2. (Page 319.) Nucleotide sequences and deduced amino acid sequences of the V_L of the four anti-TCS mAb and of their germ-line counterparts. (a) TE2, (b) TE1, 4C8 and 2A1; same legend as Fig. 1. The positions of primers V_K-BACK and J_K-FOR are indicated. Only the amino acid sequence of V_K21D is shown; nucleic sequences of 4C8 V_L and 2A1 V_L are compared to that of TE1 V_L. Sequences of germ-line genes: V_K21A,²⁴ V_K21D,²⁵ J_K sequences.²⁶

Table 2. The characteristics of V region genes of five anti-TCS mAb

		Hybridoma cell line				
		TE1	TE2	4C8	1G4	2A1
Gene usage	V _H family	7183	7183	36–60	J558	36–60
	V _H germ-line segment	V _H 61–1P	V _H 98–3G	V _H 207	V _H 205·12	V _H 207
	D germ-line segment	DSP2·6–DSP2·3	DFL16·2	DSP2·9c–DFL16·2c	DQ52c	DFL16·1e
	J _H germ-line segment	J _H 4	J _H 3	J _H 3	J _H 2	J _H 3
	V _L family	V _κ 21	V _κ 21	V _κ 21	—	V _κ 21
	V _L germ-line segment	V _κ 21D	V _κ 21A	V _κ 21D	—	V _κ 21D
	J _L germ-line segment	J _κ 1	J _κ 1	J _κ 1	—	J _κ 1
CDR3 length (amino acids)	Heavy chain	14	6	9	8	10
	Light chain	10	9	9	—	9
Number of mutations (nt)	V _H	2	1	2	13	3
	V _L	—	2	—	—	—
Number of mutations (amino acids)	V _H	0	1	2	9	3
	V _L	0	2	2	—	1

nt, nucleotides.

the V_H207 gene segment from the 36–60 family; 1G4 used the V_H205·12 segment from the J558 family. Various D_H (DSP2, DFL16 and DQ52) and J_H (J_H2, J_H3 and J_H4) genes were used equally by the five heavy chains, with a high occurrence of mutations in the D segments. The V gene usage in light chains was quite different from the diverse usage shown in heavy chains. They all used the gene segments from one gene family, V_κ21, and also used the same J_κ gene segment, J_κ1. Although the four light chains all used V segments from the V_κ21 family, they used different germ-line genes. The light chain of TE2 used V_κ21A, while TE1, 4C8 and 2A1 used V_κ21D. The frequencies of mutations in these anti-TCS antibodies were fairly low except for 1G4, which had nine replacement mutations in its V_H region.

Comparison of the homology of the heavy and light chains of the antibodies

Competitive binding assays indicated that the five mAb all bound to similar or related sites on TCS molecule, but only three of them, TE1, 4C8, 2A1, had cross-reactive idiotypes, because they all could be recognized by AId6C5. Thus we compared the homology of the variable region of the five mAb in order to determine their relationship (Table 3). The results showed that V genes of light chains were much more conserved than that of the heavy chains. For three mAb with the cross-reactive idiomorph AId6C5, TE1, 4C8,

2A1, the homology among V genes of heavy chains varied significantly from 64·7% to 95·2%. However, the V genes of light chains were highly homologous, with variations within 98·2–99·4%. A comparison of the deduced amino acid sequences of the V_L of the antibodies is shown in Fig. 3. These sequences were of high homology.

DISCUSSION

This study shows that the V gene usage in heavy chains is quite diverse, while the light chains of anti-TCS mAb are highly conserved. The anti-TCS mAb used the same V_κ gene family (V_κ21) and the same J_κ segment (J_κ1). It is postulated that the light chain not only associates with the heavy chain to form the complete antibody structure, but also plays a more important role in antigen binding. Lotscher *et al.*²⁷ studied the relationship between fine specificities of anti-phosphocholine antibodies and VJ usage of light chains. They found that antibodies with different specificities used different V_κ–J_κ genes, which suggested that light chains had a strong influence on specificity. Buenafe *et al.*²⁸ also indicated that, on contact with antigens with aromatic structures, a certain light chain gene family (V_κ1) played an important role in determining specificity. Furthermore, analysis of the relationships between the homology of the V_L genes and the cross-reactive idiomorph AId6C5 indicated that the three mAb (TE1, 4C8, 2A1) recognized by AId6C5 displayed higher homology among themselves than observed with TE2. The heavy chain sequences of the three AId6C5⁺ mAb (TE1, 2A1, 4C8) showed few common amino acids, while the light chain sequences shared 19 amino acids that were different from TE2

Table 3. Comparison of homology of the V region of heavy and light chains of the five anti-TCS mAb (%)

	V _H	TE1	TE2	4C8	1G4	2A1
V _L						
TE1			86·4	64·7	66·1	67·2
TE2		90·7		68·1	73·2	65·8
4C8		98·2	91·0		62·4	95·2
1G4		—	—	—		64·4
2A1		98·8	91·6	99·4	—	

Table 4. The first two predicted determinants

		+1	+1	−1	+1	Net charge
Determinant 1	172–177	Gly-Lys-Arg-Val-Asp-Lys				+2
		+1	−1		+1	
Determinant 2	97–102	Lys-Asp-Ala-Met-Arg-Lys				+2

	FR1	CDR1	FR2	
TE1VL	DIELTQSPASLAVSLGQRATISCK	KASQ SVDYDGD SYM NW	YQQKPGQPPLLIY	53
4C8VL	DIELTQSPASLAVSLGQRATISCK	KASQ SVDYDGN SYM NW	YQQKPGQPPLLIY	53
2A1VL	DIELTQSPASLAVSLGQRATISCK	KASQ SVDYDGN SYM NW	YQQKPGQPPLLIY	53
TE2VL	DIELTQSPASLAVSLGQRATISCK	RASESVDNYGICFVNW	FQQKPGQPPLLIY	53
	*****	..*..*	*****	
	CDR2	FR3	CDR3	FR4
TE1VL	AASN LES G	IPARFSGSGSGTDFTLN IHPVEE EDAA TY YC	QQSNEDPT WT	FGGG 106
4C8VL	AASN LES G	IPARFSGSGSGTDFTLN IHPVEE EDAA TY YC	QQSHEDP- WT	FGGG 105
2A1VL	AASN LES G	IPARFSGSGSGTDFTLN IHPVEE EDAA TY YC	QQSNEDP- WT	FGGG 105
TE2VL	AAS NOGS G	VPARFSGSGSGTDFTSLN IHPME EDDTAM Y FC	QQS KEVP - RT	FGGG 105
	****..*	*****	..*..*	*****
TE1VL	TKLEIK	112		
4C8VL	TKLEIK	111		
2A1VL	TKLEIK	111		
TE2VL	TKLEIK	111		

Figure 3. Comparison of the deduced amino acid sequences of the four anti-TCS light chains. Completely conserved amino acid residues are indicated (*). Comparatively conserved amino acid residues are indicated by (●). Bold face characters indicate amino acid residues shared by the three mAb recognized by Aid6C5.

(Fig. 3). Some of these shared amino acids (especially the seven common amino acids in CDR1) may possibly provide the structural basis of the cross-reactive idiotype recognized by Aid6C5.

Previous competitive antibody-binding ELISA have suggested that the five mAb might recognize similar or related determinants. Calculations performed using the ANTIGEN program of PC/GENE (IntelliGenetics Inc., Mountain View, CA) indicated that there are three possible antigenic determinants on the TCS molecule. The first two predicted determinants, with 100% and 67% probability, respectively, are as shown in Table 4.

On the other hand, use of CHARGPRO program in PC/GENE to calculate the charge properties of CDR and the pI value of the V regions of the four light chains and the four corresponding heavy chains, indicated that the net charge of three CDR varied from 0 to -5 in the light chains and 0 to +3 in the heavy chains. The calculated pI values showed that the V region of light chains was strongly acidic, 4.23–5.59, while that of the heavy chains was strongly basic, 8.91–9.67. TCS is a basic protein with a pI value of 9.4,² and the two predicted determinants each carries two net positive charges. This result suggests that the light chains may bind to TCS through ionic bonds.

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