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The human anti-thyroid peroxidase autoantibody repertoire in Graves' and Hashimoto's autoimmune thyroid diseases

Abstract Human anti-thyroid peroxidase (TPO) autoantibodies (aAb) are generated during autoimmune thyroid diseases (AITD). Within recent years, increasing knowledge of the TPO-specific aAb repertoire, gained mainly by the use of combinatorial library methodology, has led to the cloning and sequencing of around 180 human anti-TPO aAb. Analysis of the immunoglobulin (Ig) variable (V) genes encoding the TPO aAb in the ImMunoGeneTics database (IMGT) (<http://imgt.cines.fr>) reveals major features of the TPO-directed aAb repertoire during AITD. Heavy chain VH domains of TPO-specific aAb from Graves' disease patients preferentially use D proximal *IGHV1* genes, whereas those from Hashimoto's thyroiditis are characterized more frequently by *IGHV3* genes, mainly located in the middle of the *IGH* locus. A large proportion of the anti-TPO heavy chain VH domains is obtained following a VDJ recombination process that uses inverted D genes. J distal *IGKV1* and *IGLV1* genes are predominantly used in TPO aAb. In contrast to the numerous somatic hypermutations in the TPO-specific heavy chains, there is only limited amino acid replacement in most of the TPO-specific light chains, particularly in those encoded by J proximal *IGLV* or *IGKV* genes, suggesting that a defect in receptor edit-

ing can occur during aAb generation in AITD. Among the predominant *IGHV1* or *IGKV1* TPO aAb, conserved somatic mutations are the hallmark of the TPO aAb repertoire. The aim of this review is to provide new insights into aAb generation against TPO, a major autoantigen involved in AITD.

Keywords Thyroid peroxidase · Autoantibody · Phage display · Variable gene · IMGT database

Introduction

The anti-thyroid peroxidase (TPO) autoantibodies (aAb) are the most frequently represented aAb in the sera of patients suffering from autoimmune thyroid disease (AITD); they are present in 90% of Hashimoto's thyroiditis and 75% of Graves' disease patients (Mariotti et al. 1990). In vitro cytotoxic effector functions mediated by TPO-specific aAb, such as C3 complement activation (Chiovato et al. 1993; Parkes et al. 1994; Wadeux et al. 1989) and antibody-dependent cell cytotoxicity (Bogner et al. 1995; Guo et al. 1997; Metcalfe et al. 1997; Rodien et al. 1996; Weetman et al. 1989), trigger thyroid cell destruction. Moreover, it has been suggested that thyroid-infiltrating B lymphocytes as antigen-presenting cells through membrane-bound anti-TPO antibodies modulate antigen processing (Guo et al. 1996; McLachlan and Rapoport 1992; Rapoport et al. 1995).

Only one human anti-TPO antibody was obtained by cell immortalization (Horimoto et al. 1992). However, McLachlan and Rapoport's group pioneered the application of combinatorial libraries to the study of aAb in thyroid diseases (Portolano et al. 1991), and a large number of human anti-TPO aAb have since been isolated by this group and others (Chazenbalk et al. 1993; Hexham et al. 1994; Jaume et al. 1994a, b; Jaume et al. 1997; McIntosh et al. 1997; Portolano et al. 1992, 1993a, b; 1995; Prummel et al. 1994a, b). In the last 2 years, about 100 anti-TPO aAb directed against immunodominant or non-immunodominant epitopes have been described

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(Chapal et al. 2000; 2001; Guo et al. 1999; Pichurin et al. 2001). Given this enlarged TPO-specific repertoire, and particularly the numerous Ig gene sequences published to date, we compiled and analyzed the genes encoding these aAb using the international ImMunoGeneTics database (IMGT) (<http://imgt.cines.fr>), an integrated information system devoted to the study of immunoglobulins, T-cell receptors, and major histocompatibility molecules of several vertebrate species (Giudicelli et al. 1997; Lefranc and Lefranc 2001).

TPO-specific heavy chain gene usage in AITD

Ig variable domain sequences encoding TPO aAb have been obtained from Fab and single chain variable fragment (scFv) combinatorial libraries, mainly derived from thyroid-infiltrating B cells of Graves' disease patients (Chapal et al. 2000; 2001; Chazenbalk et al. 1993; Jaume et al. 1994a, b, 1997; Portolano et al. 1992, 1993a, b, 1995; Prummel et al. 1994a, b). Only two libraries constructed from thyroid-infiltrating B cells or lymph node B lymphocytes of Hashimoto's patients have been described (Hexham et al. 1994; McIntosh et al. 1997). Although we cannot formally exclude that differences observed in *IGV* gene usage of TPO-specific aAb obtained from the libraries cited in Table 1 (consisting of parts a, b and c) are due to preferential primer amplification of certain *IGV* genes or gene families, we consider that the data reflect the reality in vivo since the analyses were carried out on more than 180 human anti-TPO aAb obtained from four laboratories that used different primers. Analysis of the heavy chain variable domains of the anti-TPO aAb shows a restriction in the *IGHV* gene usage in both Graves' and Hashimoto's AITD (Table 1, consisting of parts a, b and c) (McIntosh et al. 1998; McLachlan and Rapoport 2000). The heavy chains of the anti-TPO aAb are mainly encoded by genes of the *IGHV1* (75.4%) and *IGHV3* (21.2%) subgroups, with a large predominance of the *IGHV1-3* gene in thyroid diseases.

Interestingly, *IGHV* gene analysis of anti-TPO aAb from patients with Graves' disease or with Hashimoto's hypothyroiditis clearly indicates a discrimination in *IGHV* subgroup usage (Table 2). In Graves' disease, the anti-TPO aAb mainly use *IGHV1* subgroup genes (88.9%), with overrepresentation of *IGHV1-3* (50.4%) and *IGHV1-2* (25.5%). In Hashimoto's thyroiditis, the *IGHV3* subgroup (71%) is dominant among the anti-TPO aAb, with a large predominance of *IGHV3-21* (47.4%) and *IGHV3-23* (18.4%) (Table 1 (consisting of parts a, b and c) and 2). Preferential use of *IGHV4*, *IGHV5*, and *IGHV6* genes by aAb in autoimmune diseases was suggested by several studies (Dijk-Hard van et al. 1999; Melero et al. 1998; Pascual and Capra 1992; Pascual et al. 1992a, b, c; Roben et al. 1996). On the other hand, underexpression of the *IGHV1* subgroup in aAb is a very common feature in autoimmune diseases, as demonstrated for numerous autoantigens (Bona et al. 1993). The overexpression of the *IGHV3* subgroup in Hashimoto's

thyroiditis and that of the *IGHV1* subgroup in Graves' disease seems to be a characteristic of the anti-TPO aAb repertoire, and suggests that there is a skewing of *IGHV* gene usage in TPO-specific aAb in the sera of patients suffering from autoimmune thyroid diseases.

With regard to the organization of the human *IGH* locus (Fig. 1), TPO-specific aAb from patients with Graves' disease and from Hashimoto's hypothyroiditis preferentially use D proximal *IGHV1* genes and D distal *IGHV3* genes, respectively. Two different hypotheses can explain the preferential expression and/or selection of a particular *IGHV* gene: (1) selection derived from preferential rearrangement due to the gene position in the *IGH* locus and/or accessibility to the recombinase machinery and (2) functional selection based on the recognition of defined epitopes on the TPO molecule (Sasso et al. 1989). The preferential use of the D proximal *IGHV5* subgroup gene previously designated 7183 is well documented in mice (Bona et al. 1993), but the fact that genes from *IGHV* subgroups are scattered throughout the *IGH* locus (Fig. 1) does not support the "position" hypothesis. On the other hand, the fact that non-IDR (immunodominant region) TPO-specific aAb show a restricted *IGHV1-69* gene usage (Pichurin et al. 2001) argues in favor of the second hypothesis.

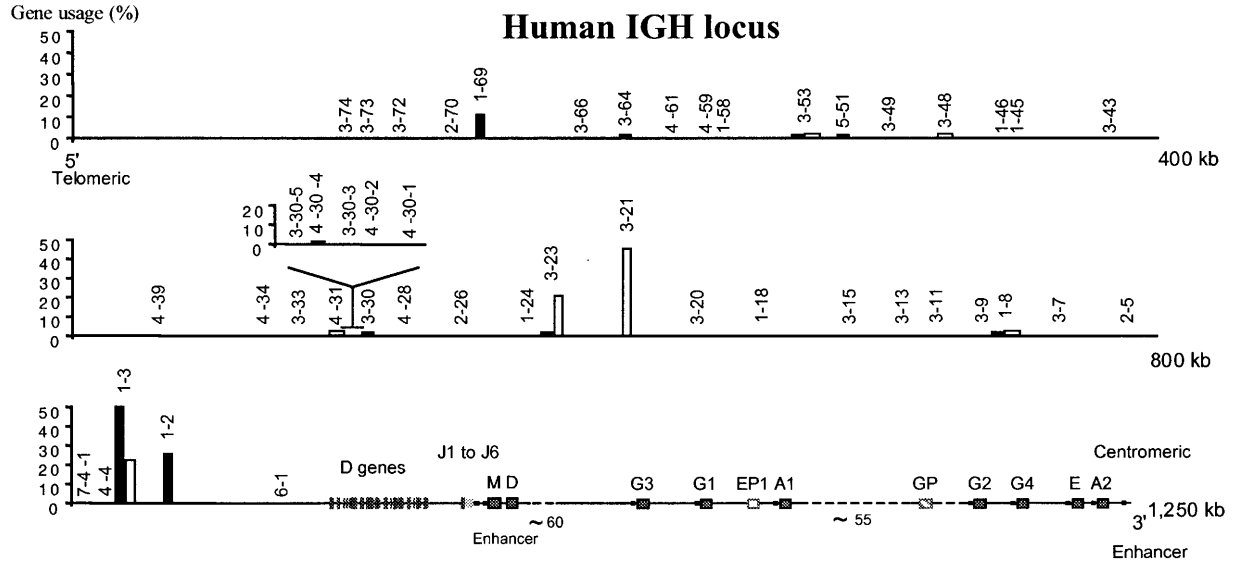
The D genes used by these aAb show a high diversity with a large number of genes in an inverted orientation of transcription (38%) (Table 1, consisting of parts a, b and c). Inverted D genes are rarely used by aAb, and this event seems to be a peculiarity of anti-TPO aAb. This observation suggests the possible involvement of particular mechanisms such as the use of D genes with irregular spacers (DIR elements) (Tuaille and Capra 1998), preferential V-D rearrangements (Tuaille and Capra 2000b), or modulation of terminal deoxynucleotidyl-transferase activity (Tuaille and Capra 2000a) to generate heavy chain diversity in the TPO repertoire. Analysis of D gene usage suggests that there is no apparent restriction in D gene use, whereas *IGHJ4* (61.6%) and *IGHJ6* (29.9%) are preferentially rearranged among the TPO-directed aAb (Tables 1 (consisting of parts a, b and c), 2) in Graves' disease.

TPO-specific light chain gene usage in AITD

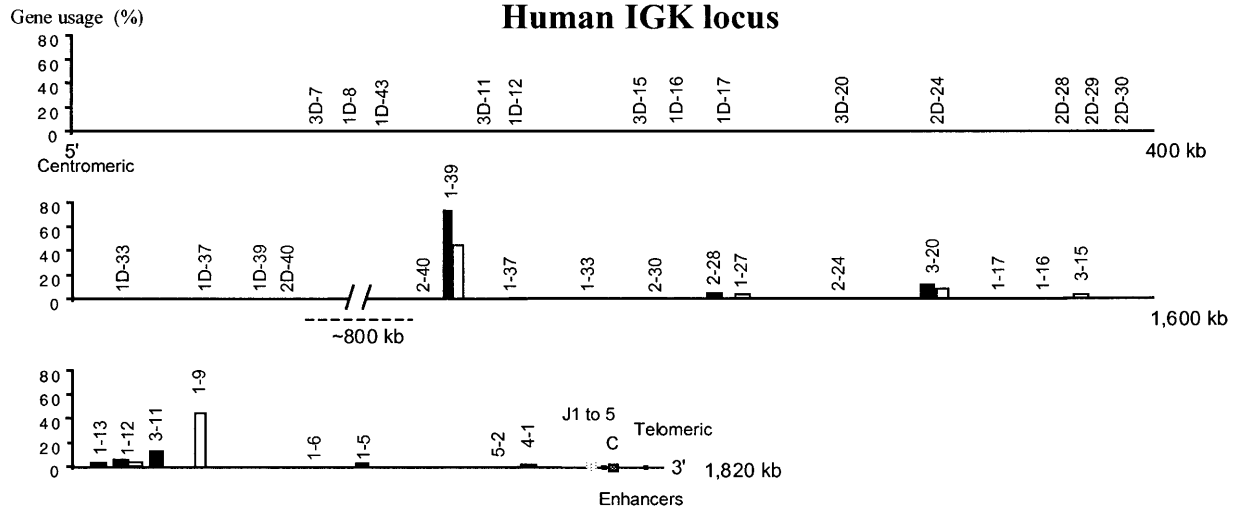
J distal *IGKV1* and *IGLV1* genes (Fig. 1) are preferentially rearranged in TPO-specific recombinant aAb (Tables 1 (consisting of parts a, b and c) and 2). Within

Fig. 1 Germline gene usage of human anti-thyroid peroxidase (TPO) antibodies in relation to their position on the immunoglobulin heavy (*IGH*), kappa (*IGK*), and lambda (*IGL*) variable gene loci. Percentage of anti-TPO clones derived from the corresponding germline gene of patients with Graves' disease (solid bars), and Hashimoto's thyroiditis (open bars). Genes *IGKV1-12* and *IGKV1-39* could not be differentiated from their duplicated genes *IGKVID-12* and *IGKID-39*, respectively. The loci representations were recovered and simplified from the IMGT database and the legend may be found at <http://imgt.cines.fr> ▶

1a



1b



1c

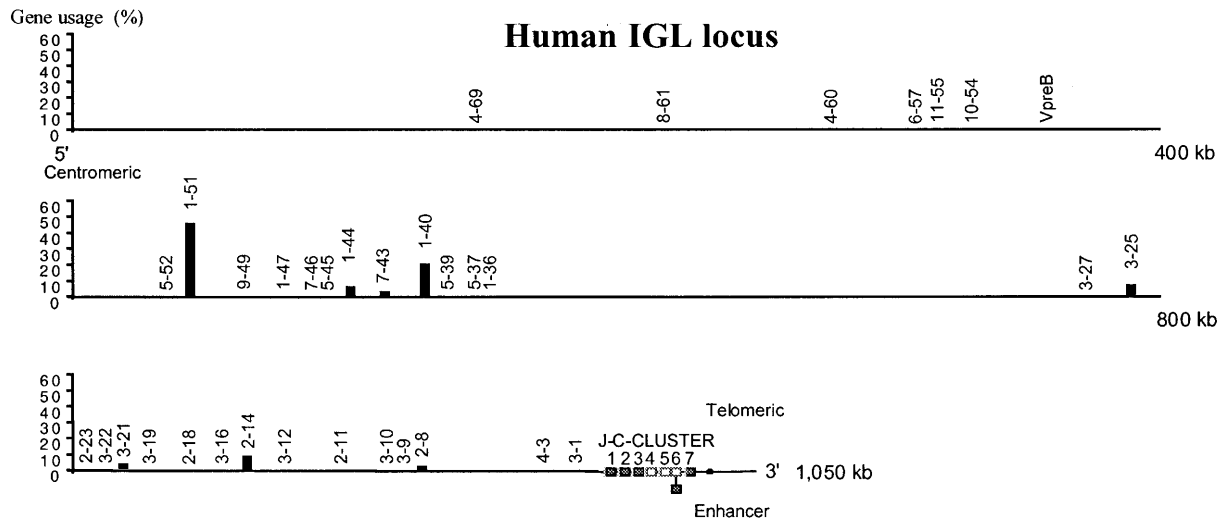


Table 1a Human anti-thyroid peroxidase (TPO) antibody fragments isolated from combinatorial libraries. Antibodies showing in-cell H/L associations are boxed

Libraries ^a	Primer specificity	Clone	Heavy chain gene ^b			Light chain gene ^b			Affinity ^c (nM)	TPO domain ^d
			IGHV	IGHD ^e	IGHU	IGKV or IGLV	IGKJ or IGLJ			
<i>Lambda phage libraries (λ-ZAP)^f</i>										
Fab from Graves' thyroid pan B cells (Portolano et al., 1991, 1992)	γ1 and κ	SP1.2	IGHV1-2*02	ND	IGHJ6*02	IGKV1/1D-39*01	IGKJ2*01	0.08	IDR/A	
		SP1.4	IGHV1-2*02	ND	IGHJ6*02	IGKV1/1D-39*01	IGKJ3*01/4*01/5*01	0.22	IDR/A1	
		SP1.5	IGHV1-2*02	ND	IGHJ6*02	IGKV1/1D-39*01	IGKJ3*01/4*01/5*01	0.06	IDR/A1	
SPI-2 IGHV x different IGKV (Roulette) (Portolano et al., 1993b)	γ1 and κ	SP1.12	kd SP1.2	kd SP1.2	kd SP1.2	IGKV1/1D-39*01	IGKJ1*01	0.09	IDR/A	
		SP1.13	kd SP1.2	kd SP1.2	kd SP1.2	IGKV1/1D-39*01	IGKJ2*01			
		SP1.14	kd SP1.2	kd SP1.2	kd SP1.2	IGKV1/1D-39*01	IGKJ2*01			
		SP1.16	kd SP1.2	kd SP1.2	kd SP1.2	IGKV1/1D-39*01	IGKJ2*01			
		SP1.17	kd SP1.2	kd SP1.2	kd SP1.2	IGKV1/1D-39*01	IGKJ2*01			
		SP1.18	kd SP1.2	kd SP1.2	kd SP1.2	IGKV1/1D-39*01	IGKJ2*01			
SPI-2 IGKV x different IGHV (Roulette) (Portolano et al., 1993b)	γ1/γ4 and κ	SP4.6	IGHV1-2*02	IGHD2-2*01inv/02inv/03inv	IGHJ4*02	kd SP1.2	kd SP1.2	0.15	IDR/A	
		SP1.7	IGHV1-2*02	ND	IGHJ6*02	kd SP1.2	kd SP1.2	IDR/A		
		SP1.9	IGHV1-2*02	ND	IGHJ6*02	kd SP1.2	kd SP1.2	IDR/A		
Fab from Graves' thyroid pan B cells (Chazanbali et al., 1993)	γ1 and κ	WR1.7	IGHV1-3*01	IGHD6-13*01	IGHJ4*02	IGKV1/1D-39*01d	IGKJ1*01d	0.2	IDR/A2	
		WR1.9	IGHV1-3*01	IGHD6-13*01	IGHJ4*02	IGKV1/1D-39*01d	IGKJ1*01d			
Fab from Graves' thyroid pan B cells (Chazanbali et al., 1993)	γ4 and κ	WR4.2	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2 ^h	0.31	IDR/A	
		WR4.3	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2 ^h			
		WR4.4	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2*01			
		WR4.5	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2*01			
		WR4.7	—	—	—	IGKV1/1D-39*01	IGKJ1*01			
		WR4.8	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2*01			
		WR4.9	—	—	—	IGKV1/1D-39*01	IGKJ1*01			
		WR4.10	IGHV1-2*02/4	IGHD2-2*01inv/2inv/3inv	IGHJ4*03	IGKV1/1D-39*01	IGKJ2*01			
		WR4.12	IGHV1-2*02/4	IGHD2-2*01inv/2inv/3inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01			
		WR4.21	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2*01			
		WR4.22	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2*01			
		WR4.25	IGHV1-2*02/4	IGHD2-2*01inv/2inv/3inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01			
		WR4.27	IGHV1-2*02/4	IGHD2-2*01inv/2inv/3inv	IGHJ4*02	IGKV1/1D-39*01 ⁱ	IGKJ2*01 ⁱ			
		WR4.28	IGHV1-2*02/4	IGHD2-2*01inv/2inv/3inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01			
		WR4.31	IGHV1-2*02/4	IGHD2-2*01inv/2inv/3inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01			
		WR4.32	IGHV1-2*02	IGHD2-2*01inv/2inv/3inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01			
		WR4.33	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2*01			
WR4.34	IGHV1-2*02	IGHD2-2*01inv/2inv/3inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01					
WR4.35	IGHV1-2*02	IGHD2-2*01inv/2inv/3inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01					
WR4.36	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2*01					
WR4.37	IGHV1-2*02	IGHD2-2*01inv	IGH4 ^g	IGKV1/1D-39*01	IGKJ2*01					
Fab from Graves' thyroid pan B cells (Chazanbali et al., 1993)	γ1 and κ	TR1.3	IGHV3-53*01	IGHD6-6*01inv	IGHJ6*03	IGKV1/1D-39*01	IGKJ1*01	0.51±0.01	IDR/A/B	
		TR1.5	IGHV3-53*01	IGHD6-6*01inv	IGHJ6*03	IGKV1/1D-39*01	IGKJ2*01		IDR/A/B	
		TR1.6	IGHV1-69*06	IGHD6-13*01inv/5-12*01inv	IGHJ3*01/2	IGKV2/2D-28*01	IGKJ2*01		IDR/B1	
		TR1.8	IGHV1-69*06	IGHD3-16*01	IGHJ3*01	IGKV2/2D-28*01	IGKJ2*01	0.27±0.01	IDR/B1	
		TR1.9	IGHV1-3*01	IGHD1-26*01	IGHJ4*02	IGKV1-13*02	IGKJ4*01	0.15±0.02	IDR/B2 ^j	
		TR1.10	IGHV1-3*01	IGHD3-16*01inv/1-14*01/3-3*01inv/2inv/1-20*01	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01	0.16	IDR/A	
		TR1-13	IGHV1-3 ^k	—	IGHJ4 ^g	IGKV1-13*02	IGKJ3*01			
Fab from Graves' thyroid pan B cells (Chazanbali et al., 1993)	γ1 and κ	JA1.9	IGHV1-2*02	ND	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01			
Fab from Graves' thyroid pan B cells (Jaume et al., 1997)	γ1 and κ/λ KM1	WR1.223	IGHV3-30-3*01 ^l	IGHD5-5*01 ^l	IGHJ4 ^g	IGKV4-1 ^l	IGKJ4 ^g	2.2	IDR/B	
		WR1.223	IGHV3-23*01 ^l	IGHD3-9*01inv ^l	IGHJ3 ^g	IGKV4-1 ^l	IGKJ5 ^g	0.81	IDR/B	
Fab from Graves' thyroid pan B cells (Suo et al., 1999)	γ1 and κ	G(N) 1	IGHV1-2 ^m	IGHD3-3/2-2 ^m	IGHJ6 ^g	IGKV3-11 ^g	—			
		G(N) 2	IGHV1-3 ^m	ND	IGHJ4 ^g	IGKV1/1D-39*01 ⁿ	—			
		G(N) 3	IGHV1-3 ^m	ND	IGHJ4 ^g	IGKV1/1D-39*01 ⁿ	—			
		G(N) 4	IGHV1-2 ^m	IGHD3-3/2-2 ^m	IGHJ6 ^g	IGKV3-11 ^g	—			
		G(N) 5	IGHV1-3 ^m	IGHD1-26inv/2-8inv ^m	IGHJ6 ^g	IGKV1/1D-39*01 ⁿ	—			
		G(N) 6	IGHV1-3 ^m	ND	IGHJ4 ^g	IGKV1/1D-39*01 ⁿ	—			
		G(N) 7	IGHV1-3 ^m	IGHD1-26inv/2-8inv ^m	IGHJ6 ^g	IGKV1/1D-39*01 ⁿ	—			
		G(N) 9	IGHV1-3 ^m	ND	IGHJ4 ^g	IGKV1/1D-39*01 ⁿ	—			
		G(N) 17	IGHV1-2 ^m	IGHD3-3/2-2 ^m	IGHJ6 ^g	IGKV3-11 ^g	—			
		G(N) 19	IGHV1-2 ^m	IGHD3-3/2-2 ^m	IGHJ6 ^g	IGKV3-11 ^g	—			
G(N) 22	IGHV1-2 ^m	IGHD3-3/2-2 ^m	IGHJ6 ^g	IGKV3-11 ^g	—					
<i>Filamentous phage libraries (phage display)^o</i>										
Fab from Graves' thyroid pan B cells (Portolano et al., 1993c)	γ1 and κ	TR1.21	IGHV1-2*02	IGHD3-16*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ4*01	0.35±0.11		
		TR1.22	IGHV1-2*02	IGHD5-18*01inv/5-5*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01		IDR/A	
		TR1.23	IGHV1-3*01	IGHD5-24*01	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01	0.54±0.15	IDR/A	
		TR1.32-1.33	IGHV3-53*01	IGHD4-11*01inv/4-4*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ4*01		IDR/A/B	
		TR1.37	IGHV1-69*06	IGHD1-20*01/1-1*01	IGHJ3*01	IGKV2/2D-28*01	IGKJ2*01	0.30	IDR/B	
Fab from Hashimoto's thyroid pan B cells (Hesham et al., 1994)	γ1 and κ	6 F	IGHV1-8*01	IGHD6-25*01/1inv/3-10*01/3-3*01/02	IGHJ6*02	IGKV1/1D-39*01	IGKJ2*01	80	as 2G4	
		7 F	IGHV4-31*01	IGHD3-10*01	IGHJ4*02	IGKV3-20*01	IGKJ1*01	80	not 2G4	
		10I	IGHV3-23*01	IGHD3-3*01/2	IGHJ6*02	IGKV1/1D-39*01	IGKJ3*01	9.3	not 2G4	
Fab from Graves' thyroid pan B cells (Hummel, 1994; Portolano, 1995)	γ1 and λ	TR1.41	IGHV1-69*01	IGHD3-10*01	IGHJ3*02	IGLV3-21*01	IGLJ1*01	0.8	IDR/B	
		WR1.102	IGHV3-23 ^p	IGHD3-22*01 ^p	IGHJ4 ^g	IGLV2-14 ^g	IGLJ2*01 ^q	2	IDR/B	
		WR1.107	IGHV1-2 ^m	IGHD5-5*01 ^p	IGHJ6 ^g	IGLV3-25 ^g	IGLJ2*01 ^q	100	IDR/B	
		WR1.112	IGHV4-30-4 ^r	ND	IGHJ4 ^g	IGLV3-25 ^g	IGLJ2*01 ^q	100		

Table 1b

Table 1 (continued)

Libraries	Primer specificity	Clone	Heavy chain gene			Light chain gene		Affinity (nM)	TPO domain
			IGHV	IGHD	IGHJ	IGKV or IGLV	IGKJ or IGLJ		
<i>Filamentous phage libraries (phage display)</i>									
Fab from Hashimoto's γ 1 and κ / λ thyroid pan B cells (McIntosh et al., 1997)		126A	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*01/2	IGKV1-9*01	IGKJ4*01		
		126B	IGHV3-21*01/2	IGHD5-12*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		IDR/B
		126C	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126D	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*02	IGKV1-12*01/02	IGKJ4*01	0.2	
		126E	IGHV3-21*01/2	IGHD1-7*01/1-20*01	IGHJ5*01/2	IGKV1-9*01	IGKJ4*01		
		126G	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*01/2	IGKV1-9*01	IGKJ5*01	0.2-3.1	IDR/B
		126H	IGHV3-21*01/2	IGHD4-23*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01	0.2	IDR/B
		126I	IGHV3-21*01/2	IGHD5-12*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126J	IGHV3-21*01/2	IGHD3-16*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126FO1	IGHV1-3*01	IGHD2-2*01inv/3inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ5*01	3.9	IDR/A
		126FO2	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ4*01	0.4-2.4	
		126FO3	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ4*01	0.4-2.4	
		126FO6	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ4*01	0.4-2.4	
		126FO8	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*02	IGKV1-9*01	IGKJ5*01	0.2-3.1	
		126FO9	IGHV3-21*01/2	IGHD2-21*01	IGHJ5*02	IGKV1-27*01	IGKJ4*01	0.094-10	
	126FO10	IGHV3-21*01/2	IGHD3-16*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01	0.094-10		
	126FO15	IGHV3-21*01/2	IGHD5-12*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01	0.094-10		
Fab from Hashimoto's γ 1 and κ / λ lymph node pan B cells (McIntosh et al., 1997)		126FP1	IGHV3-21*01/2	IGHD3-16*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126FP5	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01		IDR/A
		126FP6	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126FP7	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*01/2	IGKV1-9*01	IGKJ4*01		
		126FP8	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126FP9	IGHV1-3*01	IGHD6-6*01inv/3-16*01/3-10*01/2	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01		
		126FP10	IGHV3-21*01/2	IGHD3-16*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126FP13	IGHV1-3*01	IGHD2-2*01inv/3inv	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01	2.8	
		126FP14	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01		
		126FP15	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01	3.1	
		131TP2	IGHV3-23*01	IGHD6-6*01inv/4-23*01inv/1-26*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ3*01	3.1-4.4	
		131TP5	IGHV3-23*01	IGHD6-6*01inv/4-23*01inv/1-26*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ1*01	2.2-15	IDR/A
		131TP6	IGHV3-23*01	IGHD6-6*01inv/4-23*01inv/1-26*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ3*01	3.1-4.4	IDR/A
		131TP7	IGHV3-23*01	IGHD6-6*01inv/4-23*01inv/1-26*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ1*01	2.2-15	IDR/A
		131TP8	IGHV3-23*01	IGHD6-6*01inv/4-23*01inv/1-26*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ1*01	2.2-15	
	131TP14	IGHV3-48*01	IGHD3-16*01inv/2-21*01inv/2inv/2-8*01inv/2inv	IGHJ6*01	IGKV3-15*01	IGKJ3*01	2.6	IDR/B	
	131TP15	IGHV3-23*01	IGHD6-6*01inv/4-23*01inv/1-26*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ1*01	2.2-15		
mAb from Hashimoto's γ 1 and κ thyroid pan B cells (Homoto et al., 1992)		2G4	IGHV3-53*01/2	IGHD6-13*01/6-6*01	IGHJ4*02	IGKV3-20*01	IGKJ5*01	2.5	
Fab from Graves' thyroid pan B cells (select on denature TPO) (Guo et al., 1999; Rahimi et al., 2001)	γ 1 and κ	DN4	IGHV1-69*01/6	IGHD3-10*01	IGHJ6*02	IGKV1/1D-39*01	IGKJ1*01	NM	non-IDR
		DN 7	IGHV1-3 ⁹	IGHD1-26inv/2-8inv ⁹	IGHJ6 ⁹	IGKV1/1D-39*01 ⁹ - ⁹			IDR
		DN 8	IGHV1-3 ⁹	IGHD1-26inv/2-8inv ⁹	IGHJ6 ⁹	IGKV1/1D-39*01 ⁹ - ⁹		0.15	IDR
		DN 14	IGHV1-3 ⁹	IGHD3-3/2-2 ⁹	IGHJ6 ⁹	IGKV3-11 ⁹ - ⁹		0.26	IDR
		DN 15	IGHV1-3 ⁹	IGHD1-26inv/2-8inv ⁹	IGHJ6 ⁹	IGKV1/1D-39*01 ⁹ - ⁹			IDR
		DN 16	IGHV1-3 ⁹	IGHD1-26inv/2-8inv ⁹	IGHJ4 ⁹	IGKV1/1D-39*01 ⁹ - ⁹		0.12	IDR
	DN 20	IGHV1-3 ⁹	ND	IGHJ4 ⁹	IGKV1/1D-39*01 ⁹ - ⁹			IDR	
Fab from Graves' thyroid pan B cells (Guo et al., 1999)	γ 1 and κ	N 2	IGHV1-3 ⁹	ND	IGHJ4 ⁹	IGKV1/1D-39*01 ⁹ - ⁹			IDR
		N 5	IGHV1-3 ⁹	ND	IGHJ4 ⁹	IGKV1/1D-39*01 ⁹ - ⁹			IDR
		N 6	IGHV1-3 ⁹	IGHD3-3/2-2 ⁹	IGHJ6 ⁹	IGKV3-11 ⁹ - ⁹			IDR
		N 8	IGHV1-3 ⁹	ND	IGHJ4 ⁹	IGKV1/1D-39*01 ⁹ - ⁹			IDR
		N 11	IGHV1-3 ⁹	ND	IGHJ4 ⁹	IGKV1/1D-39*01 ⁹ - ⁹			IDR
		N 12	IGHV1-3 ⁹	ND	IGHJ4 ⁹	IGKV3-20 ⁹ - ⁹			IDR
In-cell scFv from Graves' thyroid CD19 ⁺ B cells (Chapal et al., 2000)	γ 1 and κ / λ	ICA1	IGHV1-3*01	IGHD3-3*01inv/3-9*01inv	IGHJ4*02	IGLV1-51*01	IGLJ1*01	4.17	I
		ICA5	IGHV1-69*01	IGHD3-3*01	IGHJ4*02	IGLV1-40*02	IGLJ2*01/3*01	1.82	II
		ICB7	IGHV1-3*01	IGHD3-3*01inv/3-9*01inv/4*03	IGHJ3*01/2	IGLV1-51*01	IGLJ1*01	1.20	III
scFv from Graves' thyroid CD19 ⁺ B cells (Chapal et al., 2001)	γ 1 and κ / λ	A1	IGHV1-3*01	IGHD3-16*01/5-24*01	IGHJ4*02/3	IGLV1-51*01	IGLJ2*01/3*01		
		A2	IGHV1-3*01	IGHD3-16*01	IGHJ4*02/3	IGLV1-51*01	IGLJ2*01/3*01	4.89	III
		A3	IGHV1-3*01	IGHD7-27*01	IGHJ4*02	IGLV1-51*01	IGLJ3*02		
		A4	IGHV1-3*01	IGHD5-24*01/3*01/2	IGHJ4*02	IGLV2-14*01	IGLJ2*01		
		A5	IGHV1-3*01	IGHD4-17*01/4-23*01	IGHJ4*02/3	IGLV1-51*01	IGLJ2*01/3*01		
		A6	IGHV1-3*02	IGHD7-27*01inv	IGHJ4*02/3	IGLV1-40*02	IGLJ1*01		
		A7	IGHV1-3*01	IGHD3-3*01inv/3-9*01inv	IGHJ4*02/3	IGLV1-51*01	IGLJ2*01/3*01		
		A8	IGHV3-30*04	IGHD4-23*01	IGHJ4*02	IGLV1-44*01	IGLJ2*01/3*01		
		A9	IGHV3-64*01	IGHD2-15*01inv	IGHJ6*02	IGLV1-40*01	IGLJ2*01/3*01		
		A10	IGHV1-69*01	IGHD3-3*01	IGHJ4*02	IGLV1-40*02	IGLJ1*01	5.43	IV
		A11	IGHV1-3*01	IGHD3-16*01	IGHJ4*02/3	IGLV1-51*01	IGLJ1*01	8.03	V
		A12	IGHV3-64*01	IGHD2-15*01inv	IGHJ6*02	IGLV1-51*01	IGLJ2*01/3*01	1.21	VII
		A13	IGHV1-3*01	IGHD3-3*01inv/3-9*01inv	IGHJ4*02	IGLV1-40*01	IGLJ1*01		
		A14	IGHV1-3*01	IGHD3-3*01inv/3-9*01inv	IGHJ4*02	IGLV1-40*01	IGLJ1*01		
		A15	IGHV1-3*01	IGHD3-16*01	IGHJ4*02/3	IGLV1-51*01	IGLJ1*01		
		A16	IGHV1-3*01	IGHD3-3*01inv/3-9*01inv/4*03	IGHJ3*01/2	IGLV1-51*01	IGLJ1*01		
		A17	IGHV1-69*01	IGHD3-3*01	IGHJ4*02	IGLV1-44*01	IGLJ1*01		

Table 1c

Table 1 (continued)

Libraries	Primer specificity	Clone	Heavy chain gene			Light chain gene		Affinity (nM)	TPO domain
			IGHV	IGHD	IGHJ	IGKV or IGLV	IGKJ or IGLJ		
<i>Filamentous phage libraries (phage display)</i>									
scFv from Graves' thyroid pan B cells (Chapal et al., 2001)	γ1 and κ/λ B1	B1	IGHV1-3*01	IGHD5-24*01	IGHJ4*02	IGLV1-40*02	IGLJ3*02	4.35	VI
		B2	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ4*02	IGKV1/1D-39*01	IGKJ4*01		
		B3	IGHV1-3*01	IGHD5-24*01	IGHJ4*02	IGLV7-43*01	IGLJ3*02		
		B4	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ4*02	IGLV1-51*01	IGLJ3*02		
		B5	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ1*01	IGLV1-51*01	IGLJ2*01/3*01		
		B6	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ1*01	IGLV1-51*01	IGLJ1*01		
		B7	IGHV1-3*01	IGHD2-21*01/2/3-10*01/2/3-22*01	IGHJ4*02	IGKV1D-12*01	IGKJ5*01		
		B8	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ4*02	IGLV1-51*01	IGLJ3*02		
		B9	IGHV1-3*01	IGHD2-21*01/2/3-10*01/2/3-22*01	IGHJ4*02/03	IGLV1-51*01	IGLJ3*02		
		B10	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ4*02	IGLV2-14*01	IGLJ3*02		
		B11	IGHV5-51*01	IGHD3-16*01	IGHJ4*02	IGLV1-51*01	IGLJ2*01/3*01		
scFv from Graves' thyroid TPO-purified B cells (Chapal et al., 2001)	γ1 and κ/λ T1	T2	IGHV1-3*02	IGHD2-21*01	IGHJ4*03	IGKV3-11*02	ND	5.09	IX
		T3	IGHV1-3*01	IGHD2-21*01/2/3-10*01/2/3-22*01	IGHJ4*02/3	IGKV1/1D-39*01	IGKJ4*01		
		T4	IGHV1-3*01	IGHD2-8*01inv/2nv/2-21*01inv/2nv	IGHJ4*02	IGLV2-8*01	IGLJ1*01		
		T5	IGHV1-8*01	IGHD3-3*02inv	IGHJ3*02	IGKV1-5*03	IGKJ2*01		
		T6	IGHV1-3*01	IGHD2-2*02	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01		
		T7	IGHV1-3*01	ND	IGHJ6*02	IGLV1-40*01	IGLJ2*01/3*01		
		T8	IGHV1-3*01	IGHD2-21*01/2/3-10*01/2/3-22*01	IGHJ4*02/3	IGKV1/1D-39*01	IGKJ4*01		
		T9	IGHV1-3*01	IGHD2-21*01/2/3-10*01/2/3-22*01	IGHJ4*02	IGKV1/1D-39*01	ND		
		T10	IGHV3-64*01	IGHD6-19*01	IGHJ6*02	IGKV3-11*01	IGKJ4*01		
		T11	IGHV1-3*01	IGHD2-2*02	IGHJ4*02/3	IGKV1/1D-39*01	IGKJ5*01		
		T12	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ4*01/3	IGLV1-40*02	IGLJ3*02		
		T13	IGHV1-3*01	ND	IGHJ6*02	IGLV1-40*01	IGLJ2*01/3*01/2		
		Fab from Graves' thyroid pan B cells (Pichun et al., 2001)	γ1 and κ	TF2.3	IGHV1-69*03	IGHD3-10*01	IGHJ6*02		
TF2.4	IGHV1-69*04			IGHD3-10*01	IGHJ6*02	IGKV1-12*01/2/1D-12*02	IGKJ1*01		
TF2.6	IGHV1-69*02/4/6			IGHD3-10*01	IGHJ6*02	IGKV1/1D-39*01	IGKJ1*01		
TF2.10	IGHV1-69*04			IGHD3-10*01	IGHJ6*02	IGKV1/1D-39*01	IGKJ1*01		
TF3.5	IGHV1-69*04/6			IGHD3-10*01	IGHJ6*02	IGKV3-20*01	ND		
TF3.12	IGHV1-69*04/6			IGHD3-10*01	IGHJ6*02	IGKV1-39*01/02/1/1D-39*01	IGKJ2*01		
TF3.14	IGHV1-69*04/6			IGHD3-10*01	IGHJ6*02	IGKV3-20*01	IGKJ4*01		
TF3.19	IGHV1-69*04/6			IGHD3-10*01	IGHJ6*02	IGKV3-20*01	IGKJ2*01		
T2.2	IGHV1-2*02			IGHD1-20*01inv/1-1*01inv/6-13*01/6-6*01	IGHJ6*02	IGKV3-11*01	IGKJ2*01		
T2.5	IGHV5-51*01			IGHD5-18*01/5-5*01	IGHJ6*02	IGKV1D-39*01	IGKJ4*01		
T2.6	IGHV1-3*01			IGHD5-24*01inv/5-18*01inv/5-12*01inv/5-5*01inv/3-22*01inv	IGHJ6*02	IGKV1D-39*01	IGKJ2*01		
T2.7	IGHV1-3*01			IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01		
T2.11	IGHV1-3*01			IGHD3-10*01	IGHJ4*02	IGKV3-20*01	IGKJ2*01		
T3.2	IGHV1-3*01			IGHD5-24*01inv/5-18*01inv/5-12*01inv/5-5*01inv/3-22*01inv	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01		
T3.3	IGHV1-3*01			IGHD2-21*02inv/2-15*01inv/2-2*01inv/2nv/3nv	IGHJ6*02	IGKV1/1D-39*01	IGKJ1*01		
T3.4	IGHV1-8*01			IGHD6-25*01inv/6-19*01inv/6-13*01inv/6-6*01inv/5-24*01inv	IGHJ6*02	IGKV1-12*01/2/1D-12*02	IGKJ4*01		
T3.5	IGHV1-3*01			IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01		
T3.7	IGHV1-3*01			IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ4*01		
T3.10	IGHV1-3*01			IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01		
T3.13	IGHV1-3*01			IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01		
T3.15	IGHV1-3*01	IGHD3-10*01	IGHJ4*02	IGKV3-20*01	IGKJ4*01				

^a Each library was generated from a given single patient sample except those described by Chapal et al.

^b Putative closest germline genes determined with IMGT/V-QUEST sequence alignment software (<http://imgt.cines.fr>). The nomenclature is according to the IMGT (Lefranc and Lefranc, 2001) and HUGO (Human Genome Organization) nomenclature committee (<http://www.gene.ucl.ac.uk/nomenclature>). All the germline genes or alleles presenting the same score are presented in the table.

^c Affinity measurements were performed by various techniques (Scatchard analysis, Biacore, ELISA)

^d Because of the short length of the D genes, several putative closest germline D genes have the same score of alignment.

^e TPO domains were defined by various methods (EUSA inhibition, Biacore inhibition). IDR characterized according to Chazenbalk et al. (1993) and regions I-X (Chapal et al., 2000, 2001) were determined independently.

^f All the human anti-TPO antibodies, except 2G4, were isolated from combinatorial libraries.

^g Nucleotide sequences not found in public databases. When available, information concerning the proposed germline genes is derived from the cited publications.

^h The crystal structure of TR1.9 Fab has been solved (S. Chacko et al. 1996). Residue K713 has been identified to be involved in the TPO IDR epitope recognized by the TR1.9 autoantibody (Guo et al., 2001).

ⁱ Sequence alignment by IMGT/V-QUEST and IMGT/JunctionAnalysis of ICA5 shows the same score for IGLV1-40*01 and for IGLV1-47*02.

ND: Not determined by IMGT/V-QUEST or IMGT/JunctionAnalysis.

Inv: D genes in Inverted orientation of transcription.

κt: identical to in the "roulette" studies.

NM: Not measurable

IDR: Immunodominant region

the *IGKV1* subgroup, a strong restriction is observed: 72.8% of the κ anti-TPO aAb are encoded by genes derived from the *IGKV1-39* (or *IGKVID-39*) gene in Graves' disease (Tables 1 (consisting of parts a, b and c) and 2) (McIntosh et al. 1998; McLachlan and Rapoport 2000). Concerning the TPO-specific IGL repertoire, few anti-TPO recombinant Fab expressing a λ light chain have been characterized and sequenced. This is probably due to the fact that only a few libraries have been constructed using λ -specific amplification primers (Jaume et al. 1997; McIntosh et al. 1997; Prummel et al. 1994b). The decision by other authors to use only κ -specific amplification primers for library construction was based on the fact that κ -chain TPO aAb predominated in the sera of the thyroid disease patients from whom the library originated (Chazenbalk et al. 1993; Guo et al. 1999; Hexham et al. 1994; Pichurin et al. 2001; Portolano et al. 1991, 1992, 1993a, b). Using a mixture of κ - and λ - specific primers, we recently obtained numerous λ anti-TPO scFv by an in-cell library and random combinatorial libraries (Table 1, consisting of parts a, b and c) (Chapal et al. 2000; 2001). Analysis of this enlarged λ -derived TPO repertoire revealed a dominant use of the *IGLV1* subgroup in thyroid diseases, with two genes mainly found, *IGLV1-51* (47.4%) and *IGLV1-40* (26.3%) (Tables 1 (consisting of parts a, b and c), 2). Autoantibodies with λ light chains have been described in various autoimmune diseases (Cairns et al. 1989; Prummel et al. 1994a, b; Ravirajan et al. 1998; Serrano et al. 1994; Song et al. 1998); in particular, λ anti-TSHr aAb are involved in thyroid stimulation in patients with Graves' disease (Knight et al. 1986; Williams et al. 1988; Zakarija and McKenzie 1983). Moreover, five *IGLV1-40*- and one *IGLV1-51*-derived anti-Tg aAb have been isolated from a combinatorial library constructed from a patient with Hashimoto's thyroiditis (McIntosh et al. 1996, 1998).

H/L pairing of TPO aAb

Chain pairing in a TPO-selected random library can contain in vivo H/L combinations as suggested by "roulette" studies (Costante et al. 1994; Portolano et al. 1993a). This was demonstrated by comparison of H/L combinations obtained from an in-cell library with those obtained from various random libraries (Chapal et al. 2001). However, only TPO-directed aAb from an in-cell combinatorial library (Chapal et al. 2000) and clone 2G4 obtained from cell fusion (Horimoto et al. 1992) formally reflect the in vivo situation (Table 1, consisting of parts a, b and c).

Although a previous study described the lack of promiscuity between TPO-specific heavy and light chains (Portolano et al. 1993a), an extensive analysis of H/L rearrangements of anti-TPO aAb does not show apparent restriction in H/L pairing (Table 1, consisting of parts a, b and c). Indeed, the heavy chains encoded by the dominant *IGHV1-3* gene are associated with light chains encoded by 11 of 18 different *IGKV* or *IGLV* genes (Table 1,

consisting of parts a, b and c). Reciprocally, the most frequently used light chain genes, i.e., *IGKV1-39*, *IGLV1-40*, and *IGLV1-51*, are combined with around 50% of the *IGHV* genes used by TPO aAb. Overrepresentation of *IGHV1-3/IGKV1-39*, *IGHV1-3/IGHLV1-51*, and *IGHV1-3/IGLV1-40* pairings probably reflects the predominance of the expressed *IGHV*, *IGKV*, and *IGLV* genes in the TPO antibody repertoire. The clones resulting from an in-cell library and from cell fusion show the *IGHV1-3/IGLV1-51*, *IGHV1-69/IGLV1-40*, and *IGHV3-53/IGKV3-20* associations found respectively in 14, 1, and none of the anti-TPO aAb obtained from random combinatorial libraries (Table 1, consisting of parts a, b and c). These observations indicate the need to enlarge the number of in vivo clones to definitively conclude that there is a restricted H/L pairing in TPO-specific aAb, even though it is possible to obtain at least part of the in vivo anti-TPO repertoire with combinatorial libraries.

Amino acid multi-sequence alignment of TPO-specific aAb

Whereas numerous somatic hypermutations are observed in TPO-specific heavy chains whatever the library origin (Table 3, consisting of parts a, b and c), there is no or only limited amino acid replacement in most TPO-specific light chains, particularly those encoded by the J proximal *IGLV2-14*, *IGKV1-9*, *IGKV3-11*, *IGKV3-15*, *IGKV3-20*, and *IGKV4-1* genes (Tables 1 (consisting of parts a and b), 5). The pattern of mutations in *IGHV* genes from anti-TPO aAb is typical of an antigen-driven selection during AITD. On the other hand, preferential usage of J proximal *IGLV* or *IGKV* genes for some TPO aAb, with little or no residue mutations, strongly suggests a defect in receptor editing of the light chain during aAb generation in AITD, as demonstrated for lupus-associated anti-DNA aAb (Bensimon et al. 1994; Chen et al. 1997). In this case, certain TPO-specific B cells might have been blocked in their capacity to turn off their autoreactivity by light chain replacement, leading to the acquisition of a new specificity.

As previously suggested by others (McIntosh et al. 1997; Portolano et al. 1993b, 1995) and confirmed by our recent publications (Chapal et al. 2000; 2001), extensive analysis of somatic hypermutations among *IGHV1-3*, *IGHV1-2*, and *IGKV1-39* dominant-derived aAb indicate that certain residue replacements (e.g., Ile39 and Thr95 for *IGHV1* genes) are systematically found in the majority of TPO-specific aAb independently of the library, but other amino acid mutations are mostly library or patient specific (Tables 3 (consisting of parts a, b and c), 4 (consisting of parts a and b), and 5). These observations support the hypothesis that the hypermutation process could be the hallmark of the TPO aAb repertoire.

Table 3a Amino acid sequences of human anti-TPO antibody *IGHV chains* aligned with the closest putative germline genes. Designation of the complementarity determining regions (CDR) and framework regions (FR) are according to IMGT (Lefranc and Lefranc 2001; Lefranc et al. 1999). Only substituted amino acids are shown. Antibody sequences were obtained from databases except antibodies WRI.223, KMI, WRI.102, WRI.107, and WRI.112. Boxed amino acids at the N-terminus correspond to possible primer-derived sequences

Antibody designation	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3-IMGT (105-117)	FR4-IMGT (118-129)										
X62109 IGHV1-3*01	1	20	30	40	50	60	70	80	90	100	110	111	112	118	120	130	
AF306366	QVOLLVOSGA.EVKKPKGASVKVSKAS	GYTFTSYA...	MHWVQAPQORLEWGW	INAGCNT...	KYSQKFO.GRVTITRDTTSASTAYMELSLRSEDTAVYIC	AR	GGELDYGQGTITVYSS	DPVSW...	GGELDYGQGTITVYSS	DPVSW...	GGELDYGQGTITVYSS	DPVSW...	GGELDYGQGTITVYSS	DPVSW...	GGELDYGQGTITVYSS	DPVSW...	GGELDYGQGTITVYSS
AF306367	VOL-E	S-T-S-G	V	S	S	H-T	H-T	D-I	I	D	I	D	I	D	I	D	I
AF306368	VOL-E	H-S	IN	H-S	P	V-C-Y	V-C-Y	N	T	D	T	D	T	D	T	D	T
AF306369	VOL-E	T	IN	H-S	G-Y	G	G	YL	V	D	I	I	I	I	I	I	I
AF306371	VOL-E	A-R	MLAT	T	H-P	D-R	D-R	R	R	I	N	N	T	N	T	N	T
AF306372	VOL-E	S-I-P	S-I-P	I	N	L	HG-T	F	F	NT	Y	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306373	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306374	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306375	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306376	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306377	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306378	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306379	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306380	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306381	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306382	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306383	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306384	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306385	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306386	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306387	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306388	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306389	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306390	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306391	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306392	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306393	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306394	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306395	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306396	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306397	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306398	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306399	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306400	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306401	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306402	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306403	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306404	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306405	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306406	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306407	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306408	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306409	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306410	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306411	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306412	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306413	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306414	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306415	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306416	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306417	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306418	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306419	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306420	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306421	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306422	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306423	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306424	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306425	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306426	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306427	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306428	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306429	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306430	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306431	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306432	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306433	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306434	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306435	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306436	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306437	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306438	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306439	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306440	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306441	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306442	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDYAGQGTITVYSS	LDYAGQGTITVYSS	
AF306443	VOL-E	S-I-P	S-I-P	I	N	L	H-S	F	F	Y	M	M	SPYSD	LDYAGQGTITVYSS	LDY		

Table 3b

Antibody designation	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3-IMGT (105-117)	FR4-IMGT (118-129)									
X07448 IGHV1-2*01	1	30	40	50	60	70	80	90	100	110	111	112	118	120	130	
AF306372	QVQLVQSGA.EVKKFGASVKVSKAS	GYFTFGYY	MHWVROAQQGLEWVG	INENSGGT	NYAQRKQK	GRVITRDTIS	STAYMEL	SRLSRSD	DTAVVYVC	AR	ENGLPLNT	APFYGLD	VMCGGTLVTVSS			
WR4.10	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12061	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
WR4.12	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12067	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
WR4.25	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12069	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
WR4.27	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12070	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
WR4.28	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12071	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
WR4.31	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12073	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
WR4.32	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12074	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12077	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
WR4.34	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12078	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
WR4.35	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12100	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
JAL.9	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12105	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12107	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
M82813	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
SP1.2	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
SP4.6	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
Z15084	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
WR1.107	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L22582 IGHV1-69*01	QVQLVQSGA.EVKKFGASVKVSKAS	GGTFSSVA	ISWVROAQQGLEWVG	IIPFGTA	NYAQRKQK	GRVITRDTIS	STAYMEL	SRLSRSD	DTAVVYVC	AR	VERAPEAYR	GGGTYTYS	PMDVMCGGTLVTVSS			
AF306350	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306351	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306352	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306353	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306354	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306355	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306356	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306357	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306358	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306359	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
DNA	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AF306392	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AJ238327	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
ICA5	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AJ399810	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
A10	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AJ399815	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
A17	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12094	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
TR1.6	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
TR1.8	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12086	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
L12113	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
U09084	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
M99637 IGHV1-8*01	QVQLVQSGA.EVKKFGASVKVSKAS	GYFTFSYD	INWVROAQQGLEWVG	MNPNNGT	GYAQRKQK	GRVITRDTIS	STAYMEL	SRLSRSD	DTAVVYVC	AR	ENGLPLNT	APFYGLD	VMCGGTLVTVSS			
AF306370	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
AJ399831	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
T5	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X73856	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
Z14073 IGHV3-21*01	EVQLVESGG	GLVRFGGSLRISCAAS	GYFTFSYS	QFTFSSYS	YVADSVK	GRFTISRDK	AKNSLYL	DMNSLR	AEIDTAVVYVC	AR	GGMQLQ	LNFFD	VMCGGTLVTVSS			
X98932	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126A	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98933	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126B	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98934	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126C	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98935	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126D	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98936	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126E	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98937	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126F	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98938	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126G	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98939	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126H	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98940	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126I	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98941	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126J	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98942	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126K	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98943	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126L	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98944	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126M	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98945	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126N	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98946	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126O	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
X98947	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA	A-F	GLVG	TMGLD	VMCGGTLVTVSS						
126P	VLKLEEL	N-NDP	V-W	KNA	A-AT	TS-KA										

Table 3c

Antibody designation	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3-IMGT (105-117)	FR4-IMGT (118-129)
M99660 IGHV3-23*01	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X73859 10I	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X98958 131TP2	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X98959 131TP5	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X98960 131TP6	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X98961 131TP7	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X98962 131TP8	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X98964 131TP15	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
WR1.223	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
WR1.102	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
M83134 IGHV3-30*01	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
AJ399808 A8	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
KM1	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
M99675 IGHV3-48*01	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X98963 131TP14	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
M99679 IGHV3-53*01	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
L12090 TR1.3	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
L12092 TR1.5	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
L12111 TR1.32	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X73853 264	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
M99682 IGHV3-64*01	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
AJ399809 A9	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
AJ399811 A12	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
AJ399836 T10	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
Z14238 IGHV4-30-4*01	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
WR1.112	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
L10098 IGHV4-31*01	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
X73857 7F	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
M99686 IGHV5-51*01	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
AF306373 T2.5	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ
AJ399826 B11	EVQLVESGG...GLVQPGGSLRLS	GFTFSSYA...G	MSWVROA	ISGSGGST...F-AN-DFA...	YVADSVK	GRFTISRDN	SKNTLYLQ

Table 4a Amino acid sequences of human anti-TPO antibody *IGKV* chains aligned with 2001; Lefranc et al. 1999). Only substituted amino acids are shown. Antibody sequences the closest putative germline genes. Designation of the complementary determining regions (CDR) and framework regions (FR) are according to IMGT (Lefranc and Lefranc at the N-terminus correspond to possible primer-derived sequences

Antibody designation	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3-IMGT (105-117)	FR4-IMGT (118-129)
V01577 IGKV1-12*01	1	30	40	60	80	90	110
AF306360 TF2.4	DIQMTQSPSSASVGDRTVITCRAS	QGISSSW	LAWYQKPKGAPKLLIY AAS	SLOQGVV SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANSFP	..WFQGGTKVEIKR
AF306389 TF3.4	ELV	-A-YT-	-	-	-N-	-SY-T-	..LTFGGTKVEIKR
X98967 126D	ELV	HR	-	-	-	-	..LTFGGTKVEIKR
Z00006 IGKV1-13*02 (F)	1	30	40	60	80	90	110
L12089 TR1.13	DIQMTQSPSSASVGDRTVITCRAS	QGISSSA	LA*YQKPKGAPKLLIY DAS	SLESQVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANNYP	..LTFGGTKVEIKR
L12099 TR1.9	ELVM	RG	-W-	-S-	-	-	..LTFGGTKVVDI
X63398 IGKV1-27*01	ELVM	-N-A-	-W-	-R-	-	-	..LTFGGTKVEI
X98976 126F09	DIQMTQSPSSASVGDRTVITCRAS	QGISNY	LAWYQKPKGAPKLLIY AAS	TLQSGVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QKNSAP	..LTFGGTKSEIK
Z00001 IGKV1-5*01	1	30	40	60	80	90	110
AJ399874 T5	DIQMTQSPSTLSASVGDRTVITCRAS	QGISSSW	LAWYQKPKGAPKLLIY DAS	SLESQVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANSYS	..LTFGGTKLEIKR
Z00013 IGKV1-9*01	E-VL-HP-	-VTQ-	-R-	-K-	-	-	..YTFQGGTKLEIKR
X98965 126A	DIQMTQSPFLSASVGDRTVITCRAS	QGISSSY	LAWYQKPKGAPKLLIY AAS	TLQSGVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANSYP	..LTFGGTKVEIKR
X98966 126C	V	T-D	S-N-S	S	-	-	..LTFGGTKVEIKR
X98968 126F	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98969 126G	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98970 126H	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98971 126I	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98975 126F08	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98977 126F010	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98978 126F015	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98979 126FP1	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98981 126FP6	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98982 126FP7	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X98983 126FP8	V	T	S-N-S	S	-	-	..LTFGGTKVEIKR
X17263 IGKV1D-12*01	1	30	40	60	80	90	110
AJ399871 B7	DIQMTQSPSSASVGDRTVITCRAS	QGISSSW	LAWYQKPKGAPKLLIY AAS	SLOQSGVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANSFP	..LTFGGTKVEIKR
X12691 IGRV2D-28*01	--LL-T-V-	-R-TNL-	-Q-R-	-GY-	-R-	-T-	..VLSFGGTKLEIKR
L12095 TR1.6	DIYMTQSPFLSFPVTPPEASISCRSS	QSLHNGYNY	LDMYLKPKQSPQLLIY LGS	NRASQVP DRFSGG	SGTDFLTITSISSLPEDFAFYVC	MQALQTP	..YTFQGGTK
L12097 TR1.8	EL	-G	-	-	-	-	..YTFQGGTKLE
L12114 TR1.37	EL	-F-	-	-	-	-	..YTFGHFTK
X01668 IGRV3-11*01	EIVLTQSPATLSLSPGERATLSCRAS	QSVSSY	LAWYQKPKGAPKLLIY DAS	NRATGIP ARFSGG	SGTDFLTITSISSLPEDFAFYVC	QQRSNWP	..LTYNFGGTKLEIKR
AF306380 T2.2	E	-I-N-	-	-	-	-	..LTYNFGGTKLEIKR
AJ399872 T2	-TT-	-S-	-P-	-TA-	-R-	-T-	..SFGGGLIIVLIS
AJ399878 T10	-G-	-TV-	-	-	-	-	..LTFGGTKLEIKR
M23090 IGRV3-15*01	1	30	40	60	80	90	110
X98990 131FP14	EIVMTQSPATLSLSPGERATLSCRAS	QSVSSN	LAWYQKPKGAPKLLIY GAS	TRATGIP ARFSGG	SGTDFLTITSISSLPEDFAFYVC	QOYNNWP	..LPTFFGPGTKVDIK
X12686 IGRV3-20*01	EIVLTQSPGTLISLSPGERATLSCRAS	QSVSSSY	LAWYQKPKGAPKLLIY GAS	SRATGIP DRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOYSSP	..GAFQGGTKLEIKR
AF306359 TF2.3	E	-TF-	-	-	-	-	..RYTFQGGTKLEIKR
AF306363 TF3.19	AE	-ANN-	-S	-	-	-	..LTFGGTKVEIKR
AF306364 TF3.5	E	-T-	-L-	-	-	-	..LTFGGTKVEIKR
AF306365 TF3.14	E	-F-	-	-	-	-	..LTFGGTKVEIKR
AF306379 T2.11	E	-R-	-	-	-	-	..LTFGGTKVEIKR
AF306386 T3.15	E	-A-	-T-I-	-S-	-	-	..LTFGGTKVEIKR
X73854 2G4	E	-A-	-T-S-	-T-	-	-	..LTFGGTKVEIKR
X73858 7F	E	-A-	-T-S-	-T-	-	-	..LTFGGTKVEIKR
Z00023 IGRV4-1*01	1	30	40	60	80	90	110
KM1	DIYMTQSPDLSLSPGERATLSCRAS	QSVLYSSNNKNY	LAWYQKPKGAPKLLIY WAS	TRESQVP DRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOYYSFP	..LTFGGTKVEIKR
WR1.1223	EL	-N-SRT-D-	-Q-	-	-	-	..LTFGGTKVEIKR
	..EL	-P-	-I-	-	-	-	..LTFGGTKVEIKR

Table 4b

Antibody designation	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3-IMGT (105-117)	FR4-IMGT (118-129)
X59312 IGKV1D-39*01	1	30	40	60	70	80	110
AF306358	DLQMTQSPSSLSASGDRVTITCRAS	QSISSY	LNWYQQRKAPKLLIY	AAS	SLQSGVP.SRFSGSG.SGTDFTLTISSLPEDFATYYC	QOQSYSTP	HTFGQGTKEIKR
AF306361	ELV	P-S-T	F	F	N-I	I	HTFGQGTKEIKR
AF306362	ELV	Q	V-R	F	G-A	F	HTFGQGTKEIKR
AF306381	ELV	Q	S	F	N	N	HTFGQGTKEIKR
AF306382	ELV	N	S	F	G-F	F	HTFGQGTKEIKR
AF306383	ELV	V	V	GS	N	N	HTFGQGTKEIKR
AF306384	ELV	G	N	N	E	A	HTFGQGTKEIKR
AF306385	ELV	Q	R	F	E	R	HTFGQGTKEIKR
AF306387	ELV	Q	H	H	E	R	HTFGQGTKEIKR
AF306388	ELV	E	V	GT	N	N	HTFGQGTKEIKR
AF306390	ELV	T	F	F	E	K	HTFGQGTKEIKR
AF306391	ELV	T	F	F	E	K	HTFGQGTKEIKR
AF306393	ELV	DL	T	N	FE	R	HTFGQGTKEIKR
AJ399870	ELV	T	N	N	N	N	HTFGQGTKEIKR
AJ399873	ELV	Y	G	V	V	V	HTFGQGTKEIKR
AJ399875	ELV	Y	G	V	V	V	HTFGQGTKEIKR
AJ399876	ELV	Y	G	V	V	V	HTFGQGTKEIKR
AJ399877	ELV	V	S	M	T	A	HTFGQGTKEIKR
AJ399879	ELV	HP	N	G	T	A	HTFGQGTKEIKR
WR4.10	ELV	L	H	A	N	V	HTFGQGTKEIKR
WR4.11	ELV	S	A	E	S	T	HTFGQGTKEIKR
WR4.12	ELV	I	M	S	T	L	HTFGQGTKEIKR
WR4.21	ELV	L	A	N	S	T	HTFGQGTKEIKR
WR4.22	ELV	S	H	A	N	S	HTFGQGTKEIKR
WR4.25	ELV	T	H	K	E	S	HTFGQGTKEIKR
WR4.2	ELV	T	H	A	N	S	HTFGQGTKEIKR
WR4.32	ELV	S	H	A	N	S	HTFGQGTKEIKR
WR4.33	ELV	S	H	A	N	S	HTFGQGTKEIKR
WR4.35	ELV	S	H	A	N	S	HTFGQGTKEIKR
WR4.36	ELV	S	H	A	N	S	HTFGQGTKEIKR
WR4.37	ELV	T	H	A	N	S	HTFGQGTKEIKR
WR4.3	ELV	T	H	A	N	S	HTFGQGTKEIKR
WR4.4	ELV	I	H	A	N	S	HTFGQGTKEIKR
WR4.7	ELV	N	M	S	T	L	HTFGQGTKEIKR
WR4.8	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.9	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.10	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.11	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.12	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.13	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.14	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.15	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.16	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.17	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.18	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.19	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.20	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.21	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.22	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.23	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.24	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.25	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.26	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.27	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.28	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.29	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.30	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.31	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.32	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.33	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.34	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.35	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.36	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.37	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.38	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.39	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.40	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.41	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.42	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.43	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.44	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.45	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.46	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.47	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.48	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.49	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.50	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.51	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.52	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.53	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.54	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.55	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.56	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.57	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.58	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.59	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.60	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.61	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.62	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.63	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.64	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.65	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.66	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.67	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.68	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.69	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.70	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.71	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.72	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.73	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.74	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.75	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.76	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.77	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.78	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.79	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.80	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.81	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.82	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.83	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.84	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.85	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.86	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.87	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.88	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.89	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.90	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.91	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.92	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.93	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.94	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.95	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.96	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.97	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.98	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.99	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.100	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.101	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.102	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.103	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.104	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.105	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.106	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.107	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.108	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.109	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.110	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.111	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.112	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.113	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.114	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.115	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.116	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.117	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.118	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.119	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.120	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.121	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.122	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.123	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.124	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.125	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.126	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.127	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.128	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.129	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.130	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.131	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.132	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.133	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.134	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.135	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.136	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.137	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.138	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.139	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.140	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.141	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.142	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.143	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.144	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.145	ELV	N	V	H	T	T	HTFGQGTKEIKR
WR4.146	ELV	N	V	H			

Table 5 Amino acid sequences of human anti-TPO antibody IGLV chains aligned with 2001; Lefranc et al. 1999). Only substituted amino acids are shown. Antibody sequences the closest putative germline genes. Designation of the complementary determining regions (CDR) and framework regions (FR) are according to IMGT (Lefranc and Lefranc Boxed amino acids at the N-terminus correspond to possible primer-derived sequences

Antibody designation	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	CDR3-IMGT (105-117)	FR4-IMGT (118-129)
M94116 IGLV1-40*01	1	30	40	60	80	100	120
AJ399845 A6	QSVLTQPPS.VSGAPQQRVTISCTGS	SSNIGAGDY...	VHWYQQLPGTAPKLLIY	GNS.....	NRPSGVP.DRPSGSK.SGTSASLAITGLQAEDEADYYC	QSYDSSLSG	
AJ399848 A9	V.....	S.....	F.....	F.....	H.....	H.....	DVFGTGKLEIKR
AJ399849 A10	A.....	T.....	F.....	D.....	S.....	F.....	LFVGGGKTVTLG
AJ399852 A13	V.....	T.....	S.....	D.....	A.....	F.....	YVFGTGLTVLIG
AJ399856 B1	V.....	T.....	V.....	V.....	S.....	F.....	DVFGTGKLEIKR
AJ399867 T7	V.....	A.....	V.....	D.....	V.....	F.....	RVFVGGGKLEIKR
AJ399868 T12	V.....	T.....	N.....	T.....	V.....	F.....	VAFVGGGKLEIKR
AJ399869 T13	S.....	T.....	D.....	D.....	R.....	H.....	WFVGGGKLEIKR
Z73654 IGLV1-44*01	QSVLTQPPS.ASGTQQRVTISCTGS	SSNIGSNY...	VHWYQQLPGTAPKLLIY	SNN.....	QRPSGVP.DRPSGSK.SGTSASLAISGLQAEDEADYYC	AAMDSSLSG	
AJ399847 A8	P.....	S.....	C.....	M.....	D.....	S.....	S.....
AJ399855 A17	[SV].....	A.....	C.....	G.....	D.....	S.....	FVFGGKTVTLG
D87016 IGLV1-47*02	QSVLTQPPS.ASGTQQRVTISCTGS	SSNIGSNY...	VHWYQQLPGTAPKLLIY	RNN.....	QRPSGVP.DRPSGSK.SGTSASLAISGLQAEDEADYYC	AAMDSSLSG	
AJ238330 ICA5	V.....	T.....	H.....	G.....	N.....	Q.....	N.....
Z73661 IGLV1-51*01	QSVLTQPPS.VSAAQQRVTISCTGS	SSNIGNNY...	VSWYQQLPGTAPKLLIY	DNM.....	KRPSGIP.DRPSGSK.SGTSATLGTGLQAEDEADYYC	GTWDDSSLSA	
AJ238329 ICA1	R.....	S.....	K.....	F.....	A.....	E.....	VFVGGKTVDLIKS
AJ238331 ICB7	V.....	T.....	L.....	T.....	E.....	T.....	YVFGTGLTVLIG
AJ399840 A1	V.....	S.....	F.....	F.....	V.....	F.....	VVFGGKTVLIG
AJ399841 A2	V.....	M.....	R.....	Q.....	V.....	F.....	LVFGGKTVDLIKR
AJ399842 A3	S.....	T.....	T.....	T.....	S.....	F.....	KVFGGKTVLIG
AJ399844 A5	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399846 A7	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399850 A11	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399851 A12	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399853 A15	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399854 A16	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399858 B4	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399859 B5	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399860 B6	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399861 B8	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399862 B9	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399864 B11	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
AJ399865 T1	V.....	T.....	T.....	T.....	S.....	F.....	LVFGGKTVLIG
Z73664 IGLV2-14*01	QSAITQPPS.VSGSPQSVTISCTGT	SSDVGGNY...	VSWYQQLPGTAPKLLIY	EVS.....	NRPSGVS.NRPSGSK.SGNTASLITSLGQAEDEADYYC	SSYTSSTL	
AJ399843 A4	V.....	T.....	R.....	I.....	Y.....	G.....	T.....
AJ399863 B10	V.....	T.....	R.....	I.....	Y.....	G.....	T.....
WR1.107	V.....	T.....	R.....	I.....	Y.....	G.....	T.....
WR1.112	V.....	T.....	R.....	I.....	Y.....	G.....	T.....
X97462 IGLV2-8*01	QSAITQPPS.ASGSPQSVTISCTGT	SSDVGGNY...	VSWYQQLPGTAPKLLIY	EVS.....	NRPSGVP.DRPSGSK.SGNTASLITSLGQAEDEADYYC	SSYAGSNF	
AJ399866 T4	F.....	V.....	Y.....	I.....	N.....	C.....	P.....
X71966 IGLV3-21*01	SYVLTQPPS.VSVAQGTARITCGN	NIGSKS.....	VHWYQQLPGTAPKLLIY	YDS.....	DRPSGIP.ERPSGSN.SGNTATLITSRVQAEDEADYYC	QVWDDSSDH	
U09085 TR1.41	EL.....	Q.....	T.....	S.....	A.....	F.....	R.....
X97474 IGLV3-25*01	SYELMQPPS.VSVSPQPTARITCGSD	ALPKQY.....	AYWYQQLPGTAPKLLIY	KDS.....	ERPSGIP.ERPSGSS.SGTTVTLITSLGQAEDEADYYC	QASDSSGTY	
WR1.107	V.....	T.....	H.....	T.....	V.....	R.....	V.....
WR1.112	V.....	T.....	H.....	T.....	V.....	R.....	V.....
X14614 IGLV7-43*01	QTVVLTQPPS.LTVSPGTVTLTCCAS	TGAVTSGY...	PMWFOQPGQAPRALIY	STS.....	NKHSWTP.ARFSGSL.LGKKAALTLGVOPEDEAEYIC	LLYVGGAO	
AJ399857 B3	A.....	P.....	N.....	N.....	R.....	F.....	V.....

Correlation between Ig gene usage and TPO-specific antibody epitopes

Pairing of one defined heavy chain with different light chains does not alter antigen binding (Burton and Barbas 1992, 1994). This observation strongly suggests that the heavy chain initiates the formation of the antigen/antibody complex and thereby provides the specificity of the interaction, whereas its light chain counterpart stabilizes the interaction with subsequent affinity modulation (Noel et al. 1996). Such an effect of the anti-TPO aAb light chain on affinity is less conclusive, since neither *IGKV* nor *IGLV* gene usage of anti-TPO aAb has been shown to modulate antigen affinity (Chapal et al. 2000, 2001; McIntosh et al. 1997; Portolano et al. 1991, 1992, 1993b). On the other hand, several groups have pointed out that domain A of the TPO immunodominant region (IDR/A) is preferentially recognized by TPO-specific aAb with the *IGKVI-39* light chain, whereas TPO-specific aAb showing other *IGKV* light chains map in domain B of the IDR (IDR/B) (Table 1, consisting of parts a, b and c) (Chazenbalk et al. 1993; Costante et al. 1994; Guo et al. 1998; Jaume et al. 1996, 1997; McIntosh et al. 1997; Portolano et al. 1995). This IDR/B has been at least partially identified even though the location of the IDR on the TPO molecule is still under debate. Region 713–721 is located on the C-terminal myeloperoxidase-like domain of the TPO molecule; this region, recognized by murine Mab 47/C21 antibody (Finke et al. 1991; Libert et al. 1991) and by serum polyclonal TPO aAb (Libert et al. 1991; Ruf et al. 1989), was initially thought to be outside the IDR (Chazenbalk et al. 1993). Furthermore, mutations in the 713–721 region do not affect the recognition of aAb directed against IDR (Nishikawa et al. 1996). On the other hand, high concentrations of IDR/B-specific aAb TR1.9 inhibited the binding of Mab47/C21 to TPO (Guo et al. 1998) and mapped an epitope comprising amino acid residue K713 (Guo et al. 2001), suggesting that region 713–721 is located on the fringe of an IDR. The crystal structure of the Fab TR1.9 has been solved (Chacko et al. 1996), but in the absence of the three-dimensional structure for the complex of TR1.9 with TPO, it is difficult to determine the structural details of the binding.

The role *IGLV* genes play in affecting anti-TPO specificity remains to be elucidated. The initially described λ -derived anti-TPO aAb had low affinity and were directed against TPO-IDR/B (Portolano et al. 1995; Prummel et al. 1994b). In contrast, some of our λ -derived aAb demonstrated high affinity to TPO and inhibited the binding of a majority of the serum aAb to TPO (Bresson et al. 2001; Chapal et al. 2001), suggesting that these aAb recognized the IDR (defined by epitope mapping using BIACORE as regions II, VI, and VIII) (Table 1, consisting of parts a, b and c). Future studies involving λ -derived aAb such as T13/VI, B4/VIII, or ICA5/II and Fab defining IDR/A and /B (WR1–7, SP1–4, TR1–8, and TR1–9) could shed new light on the epitope specificity and gene usage of these aAb that recognize IDR.

Recently, Pichurin et al. (2001) produced and characterized human recombinant aAb by phage display technology binding outside the TPO-IDR (defined as non-IDR). All these heavy chains are encoded by *IGHVI-69*, with an extremely long CDR3, and paired with different types of light chains, suggesting that non-IDR specificity is determined primarily by a common heavy chain. Interestingly, almost all IDR-specific aAb obtained in the same experiment use *IGHVI-2* and *IGHVI-3*, as is also the case for a majority of the IDR aAb previously described (Table 1, consisting of parts a, b and c). Does *IGHVI-2* or *IGHVI-3* gene usage reflect a particular TPO-IDR specificity of recombinant aAb? Even though the methodologies used to define epitope recognition of anti-TPO recombinant aAb are different, these results reveal the difficulty of correlating gene usage with epitope recognition of TPO-specific aAb.

Conclusion

Several laboratories have produced and characterized numerous human anti-TPO aAb, leading to an enlarged autoantibody repertoire. Analysis of these antibodies using the IMGT database (Giudicelli et al. 1997; Lefranc 2001; Lefranc and Lefranc 2001; Lefranc et al. 1999) reveals several characteristics of the TPO-specific aAb repertoire: (1) a restriction in the *IGV* gene usage to generate anti-TPO aAb in AITD, (2) a VDJ recombination process using preferentially inverted D genes, (3) limited somatic mutations of J proximal light chain genes suggesting a defect in receptor editing in AITD, and (4) presence of certain somatic mutations systematically in the anti-TPO aAb repertoire. The annotations described in this paper and the protein display will soon be available as a new specialized IMGT page on human anti-TPO aAb genes. This page will evolve with time and integrate all the sequences devoted to autoantibodies that are published in the future.

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