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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; Wadeleux 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.

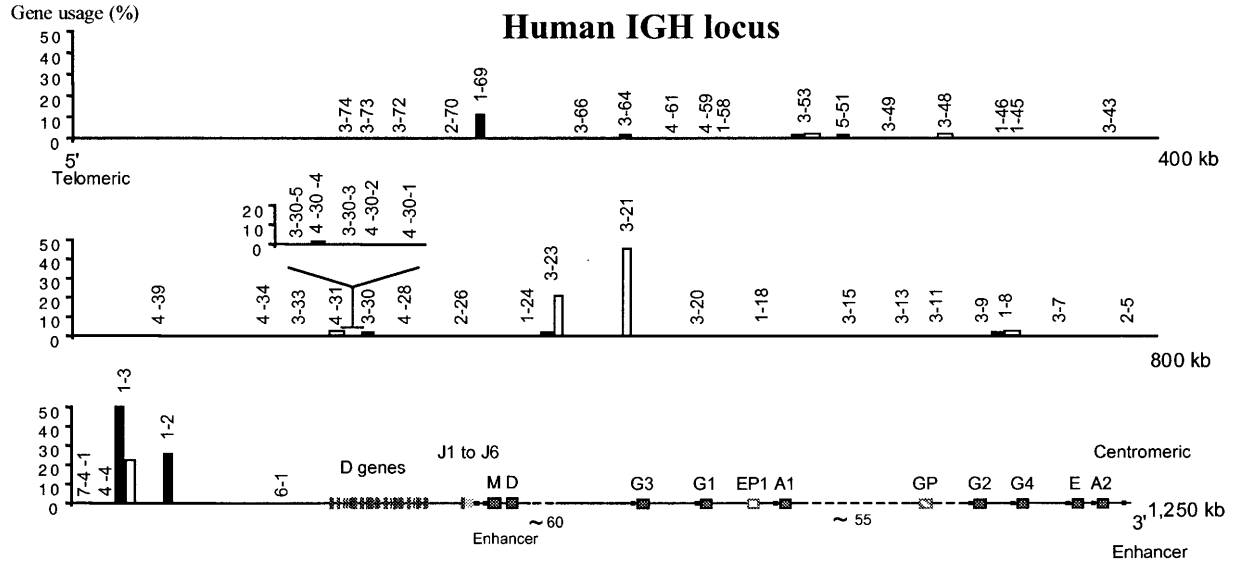
The D genes used by these aAb show a high diversity with a large number of genes in an inverted orientation of transcription (38%) (Table 1, consisting of parts a, b and c). Inverted D genes are rarely used by aAb, and this event seems to be a peculiarity of anti-TPO aAb. This observation suggests the 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

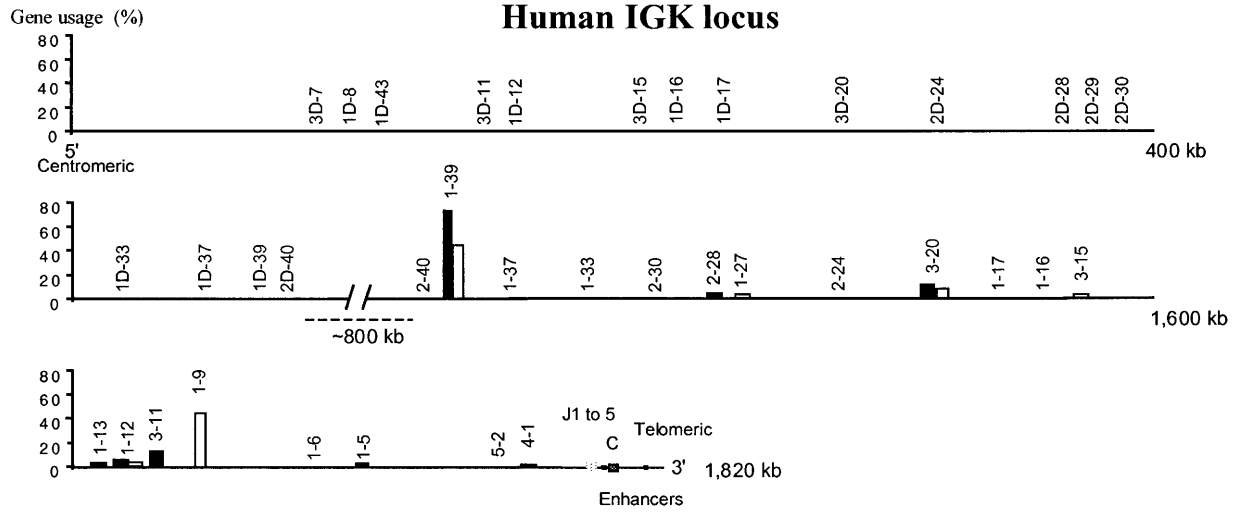
J distal *IGKV1* and *IGLV1* genes (Fig. 1) are preferentially rearranged in TPO-specific recombinant aAb (Tables 1 (consisting of parts a, b and c) and 2). Within

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

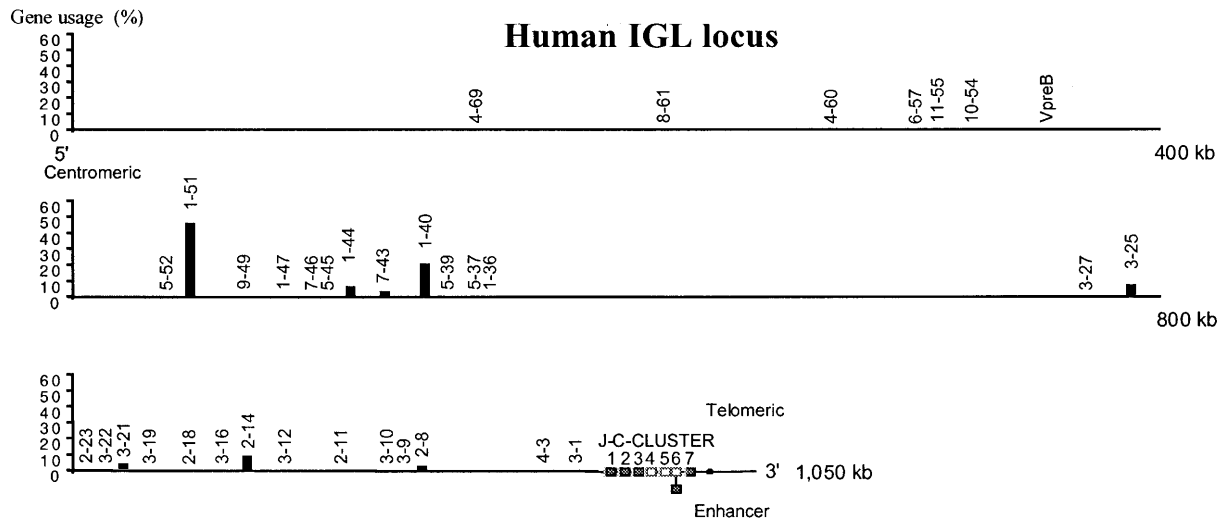


Table 1a Human anti-thyroid peroxidase (TPO) antibody fragments isolated from combinatorial libraries. Antibodies showing in-cell H/L associations are boxed

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	id SP1.2	id SP1.2	id SP1.2	IGKV1/1D-39*01	IGKJ1*01	0.09	IDR/A	
		SP1.13	id SP1.2	id SP1.2	id SP1.2	IGKV1/1D-39*01	IGKJ2*01			
		SP1.14	id SP1.2	id SP1.2	id SP1.2	IGKV1/1D-39*01	IGKJ2*01			
		SP1.16	id SP1.2	id SP1.2	id SP1.2	IGKV1/1D-39*01	IGKJ2*01			
		SP1.17	id SP1.2	id SP1.2	id SP1.2	IGKV1/1D-39*01	IGKJ2*01			
		SP1.18	id SP1.2	id SP1.2	id 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	id SP1.2	id SP1.2	0.15	IDR/A	
		SP1.7	IGHV1-2*02	ND	IGHJ6*02	id SP1.2	id SP1.2		IDR/A	
		SP1.9	IGHV1-2*02	ND	IGHJ6*02	id SP1.2	id 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	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	—	0.57	IDR/A	
		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			
		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*01	IGHD4-4*01/4-11*01	IGHJ4*02	IGLV1-51*01	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

Table 2 Germline genes used by the human TPO-specific autoantibody repertoire (ND not determined by IMGTV-QUEST)

Thyroid disease	IG variable gene usage ^a			IGHJ gene			IGKV gene			IGKJ gene			IGLV gene			IGLJ gene			
	gene	n	% ^b	gene	n	% ^b	gene	n	% ^b	gene	n	% ^b	gene	n	% ^b	gene	n	% ^b	
Graves' disease ^c	<i>IGHV1-2</i>	35	25.5	<i>IGHJ1</i>	2	1.4	<i>IGKV1-5</i>	1	0.9	<i>IGKJ1</i>	18	17.4	<i>IGLV1-40</i>	10	26.3	<i>IGLJ1</i>	13	34.2	
	<i>IGHV1-3</i>	69	50.4				<i>IGKV1-12</i>	3	2.9				<i>IGLV1-44</i>	2	5.2				
	<i>IGHV1-8</i>	2	1.4	<i>IGHJ3</i>	7.5	5.4	<i>IGKV1-13</i>	2	1.9	<i>IGKJ2</i>	38	36.9	<i>IGLV1-51</i>	18	47.4	<i>IGLJ2</i>	10	26.3	
	<i>IGHV1-69</i>	16	11.6				<i>IGKV1-39</i>	75	72.8										
	<i>IGHV3-23</i>	2	1.4	<i>IGHJ4</i>	84.5	61.6				<i>IGKJ3</i>	3	2.9	<i>IGLV2-8</i>	1	2.6	<i>IGLJ3</i>	14	36.8	
	<i>IGHV3-30</i>	2	1.4	<i>IGHJ6</i>	41	29.9	<i>IGKV2-28</i>	3	2.7	<i>IGKJ4</i>	15	14.5	<i>IGLV2-14</i>	3	7.9	ND	1	2.6	
	<i>IGHV3-53</i>	3	2.2				<i>IGKV3-11</i>	10	9.7				<i>IGLV3-21</i>	1	2.6				
	<i>IGHV3-64</i>	3	2.2	- ^d	2	1.4	<i>IGKV3-20</i>	7	6.8	<i>IGKJ5</i>	3	2.9	<i>IGLV3-25</i>	2	5.2				
	<i>IGHV4-30-4</i>	1	0.7				<i>IGKV4-1</i>	2	1.9	ND	3	2.9	<i>IGLV7-43</i>	1	2.6				
	<i>IGHV5-51</i>	2	1.4							- ^d	23	22.3							
Hashimoto's disease	<i>IGHV1-3</i>	9	23.7	<i>IGHJ4</i>	2	5.2	<i>IGKV1-9</i>	16	42.1	<i>IGKJ1</i>	5	13.1							
	<i>IGHV1-8</i>	1	2.6	<i>IGHJ5</i>	18	47.4	<i>IGKV1-12</i>	1	2.6										
							<i>IGKV1-27</i>	1	2.6	<i>IGKJ2</i>	1	2.6							
	<i>IGHV3-21</i>	18	47.4	<i>IGHJ6</i>	18	47.4	<i>IGKV1-39</i>	17	44.7	<i>IGKJ3</i>	4	10.5							
	<i>IGHV3-23</i>	7	18.4				<i>IGKV3-15</i>	1	2.6										
	<i>IGHV3-48</i>	1	2.6				<i>IGKV3-20</i>	2	5.2	<i>IGKJ4</i>	24	63.0							
	<i>IGHV3-53</i>	1	2.6						<i>IGKJ5</i>	4	10.5								
	<i>IGHV4-31</i>	1	2.6																

^a *IGHD* gene usage is not indicated since numerous anti-TPO antibody gene sequences present the same alignment score with different germline genes

^b % = $n/N \times 100$, where n = number of anti-TPO *IGHV* genes in the *IGHV* subgroup and N = total number of anti-TPO *IGHV* genes studied

^c $N=37$ for *IGHV* and for *IGHJ*; $N=103$ for *IGKV* and for *IGKJ*; $N=38$ for *IGLV* and for *IGLJ*

^d Nucleotide sequences not annotated by IMGTV-QUEST

^e $N=35$ for *IGHV* and for *IGHJ*; $N=38$ for *IGKV* and for *IGKL*

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	OVOLVOSGA.EVKKPKGASVKVSKAS	GYTFTSYA...	MHWVQAPQORLEWGW	INAGCNT...	KYSQKFO.GRVTITRDTISASTAYMELISLRSEDTAVYIC	AR	
AF306366	VOL-E	S-T-S-G	V	H-T	D-I	DPVSW	GGELDYGQGTIVTVSS
AF306367	VOL-E	H-S	IN	V-G-Y	N-N-T-D-T	KATLGA	LYGMDVWGQGTIVTVSS
AF306368	VOL-E	H-S	IN	G-Y	NL-V-D-I	KATLGA	LYGMDVWGQGTIVTVSS
AF306369	VOL-E	MIAT	T	G-Y	I-N-N-T	KVAAPAY	NFADVWGQGTIVTVSS
AF306371	VOL-E	S-I-P	I	N-L	F-T-Y-M	SPYSD	LDVWGQGTIVTVSS
AF306374	VOL-E	S-I-P	I	H-S	F-T-Y-M	SPYSD	LDVWGQGTIVTVSS
AF306375	VOL-E	S-I-P	I	H-S	F-T-Y-M	SPYSD	LDVWGQGTIVTVSS
AF306376	VOL-E	S-I-P	I	H-S	F-T-Y-M	SPYSD	LDVWGQGTIVTVSS
AF306377	VOL-E	A	I	H-T	A-T-L	RGMDVWGQGTIVTVSS	LDVWGQGTIVTVSS
AF306378	VOL-E	S-I-P	I	H-T	A-T-L	RGMDVWGQGTIVTVSS	LDVWGQGTIVTVSS
IC61	E	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
IC67	V	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ238328	E	M-S-N	I	H-T-S	L-F-S-N-R-S-L-T	DPVNG	DFDYWGQGTIVTVSS
A1	E	M-S-N	I	H-T-S	L-F-S-N-R-S-L-T	DPVNG	DFDYWGQGTIVTVSS
AJ399801	E	A-S-D	I	H-T-R	T-D-L-TT-A-T	VFTGW	VAEFDVWGQGTIVTVSS
AJ399802	E	A-S-D	I	H-T-R	T-D-L-TT-A-T	VFTGW	VAEFDVWGQGTIVTVSS
AJ399803	E	RI-E	T	H-T-R	X-E-D-V-N	DPVNG	DFDYWGQGTIVTVSS
AJ399804	E	RI-E	T	H-T-R	X-E-D-V-N	DPVNG	DFDYWGQGTIVTVSS
AJ399805	E	RI-E	T	H-T-R	X-E-D-V-N	DPVNG	DFDYWGQGTIVTVSS
AJ399806	E	RI-E	T	H-T-R	X-E-D-V-N	DPVNG	DFDYWGQGTIVTVSS
AJ399807	E	RI-E	T	H-T-R	X-E-D-V-N	DPVNG	DFDYWGQGTIVTVSS
AJ399812	A13	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399813	A14	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399814	A16	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399816	B1	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399817	B2	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399818	B3	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399819	B4	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399820	B5	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399821	B6	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399822	B7	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399823	B8	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399824	B9	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399825	B10	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399827	T1	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399828	T2	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399829	T3	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399830	T4	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399832	T6	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399833	T7	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399834	T8	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399835	T9	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399837	T11	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399838	T12	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
AJ399839	T13	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
L12087	TR1.10	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
TR1.9	WR1.9	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
L12102	WR1.7	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
L12103	WR1.9	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
L12109	TR1.23	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
X98940	X98940	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
X98941	X98941	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
X98942	X98942	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
X98943	X98943	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
X98949	X98949	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
X98953	X98953	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
X98955	X98955	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
X98956	X98956	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS
X98957	X98957	S-SI-N	I	H-T-R	V-D-S	DPNFG	DFDSWGQGTIVTVSS

Table 3b

Antibody designation	FR1-IMGT (1-26)	CDR1-IMGT (27-38)	FR2-IMGT (39-55)	CDR2-IMGT (56-65)	FR3-IMGT (66-104)	110	111	112	118	120	130
X07448 IGHV1-2*01	QVQLVQSGA.EVKKFGASVKVSKAS	GYFTFGYY	MHWVROAQQGLEWVG	INENSGGT	NYAQRKQ.GRVITRDTISITAYMELSRLSRSDTAVVYV AR						
AF306372 T2.2	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.10 L12061	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.12 L12063	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.25 L12067	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.27 L12069	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.28 L12070	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.2 L12071	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.31 L12073	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.32 L12074	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.34 L12077	VLLEEE-L	N-ND	V-W	KNA	M-S						
WR4.35 L12078	VLLEEE-L	N-ND	V-W	KNA	M-S						
JAL.9 L12100	VLLEEE-L	N-ND	V-W	KNA	M-S						
TR1.21 L12105	VLLEEE-L	N-ND	V-W	KNA	M-S						
L12107 TR1.22	VLLEEE-L	N-ND	V-W	KNA	M-S						
M82813 SP1.2	VLLEEE-L	N-ND	V-W	KNA	M-S						
SP4.6 TR1.2	VLLEEE-L	N-ND	V-W	KNA	M-S						
Z15084 WR1.107	VLLEEE-L	N-ND	V-W	KNA	M-S						
L22582 IGHV1-69*01	QVQLVQSGA.EVKKFGSIVKSKAS	GGTFSSVA	ISWVROAQQGLEWVG	IIPILFGTA	NYAQRKQ.GRVITRDTISITAYMELSRLSRSDTAVVYV AR						
AF306350 TR2.10	VLLEEE-L	N-ND	V-W	KNA	M-S						
AF306351 TR2.3	VLLEEE-L	N-ND	V-W	KNA	M-S						
AF306352 TR2.6	VLLEEE-L	N-ND	V-W	KNA	M-S						
AF306353 TR3.12	VLLEEE-L	N-ND	V-W	KNA	M-S						
AF306354 TR3.12	VLLEEE-L	N-ND	V-W	KNA	M-S						
AF306355 TR3.19	VLLEEE-L	N-ND	V-W	KNA	M-S						
AF306356 TR3.5	VLLEEE-L	N-ND	V-W	KNA	M-S						
AF306357 TR3.14	VLLEEE-L	N-ND	V-W	KNA	M-S						
AF306392 DNA	VLLEEE-L	N-ND	V-W	KNA	M-S						
AJ238327 ICA5	VLLEEE-L	N-ND	V-W	KNA	M-S						
AJ399810 A10	VLLEEE-L	N-ND	V-W	KNA	M-S						
AJ399815 A17	VLLEEE-L	N-ND	V-W	KNA	M-S						
L12094 TR1.6	VLLEEE-L	N-ND	V-W	KNA	M-S						
L12096 TR1.8	VLLEEE-L	N-ND	V-W	KNA	M-S						
L12113 TR1.37	VLLEEE-L	N-ND	V-W	KNA	M-S						
U09084 TR1.41	VLLEEE-L	N-ND	V-W	KNA	M-S						
M99637 IGHV1-8*01	QVQLVQSGA.EVKKFGASVKVSKAS	GYFTFSYD	INWVROAQQGLEWVG	MNPNSGNT	GYAQRKQ.GRVITRDTISITAYMELSRLSRSDTAVVYV AR						
AF306370 TR3.4	VLLEEE-L	N-ND	V-W	KNA	M-S						
AJ399831 T5	VLLEEE-L	N-ND	V-W	KNA	M-S						
X73856 6F	VLLEEE-L	N-ND	V-W	KNA	M-S						
Z14073 IGHV3-21*01	EVQLVESGG.GLVRFGGSLRISCAAS	GFTFSSYS	MHWVROAQQGLEWVS	ISSSSVYI	YYADSVK.GRFTISRDKANSLYLQMNLSLRLEDTAVVYV AR						
X98932 126A	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98933 126B	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98934 126C	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98935 126D	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98936 126F	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98937 126G	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98938 126H	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98939 126J	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98944 126TO8	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98945 126TO9	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98946 126TO10	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98947 126TO15	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98948 126TF1	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98950 126TF6	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98951 126TF7	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98952 126TF8	VLLEEE-L	N-ND	V-W	KNA	M-S						
X98954 126TF10	VLLEEE-L	N-ND	V-W	KNA	M-S						

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-IMG1 (1-26)	CDR1-IMG1 (27-38)	FR2-IMG1 (39-55)	CDR2-IMG1 (56-65)	FR3-IMG1 (66-104)	CDR3-IMG1 (105-117)	FR4-IMG1 (118-129)
V01577 IGKV1-12*01	1	30	40	60	80	90	110
AF306360 TF2.4	DIQMTQSPSSASVGDRTVITCRAS	QGISSSW	LAWYQKPKGAPKLLIY AAS	SLOQGVV SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANSFP	..WTFGGGKVEIKR
AF306389 TF3.4	ELV	-A-YT-	-	-	-N-	-SY-T-	..LTFGGGKVEIKR
X98967 126D	ELV	HR	-	-	-	-	..LTFGGGKVEIKR
Z00006 IGKV1-13*02 (F)	1	30	40	60	80	90	110
L12089 TR1.13	AIQLTQSPSSLSASVGDRTVITCRAS	QGISSA	LA*YQKPKGAPKLLIY DAS	SLESQVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANNYP	..LTFGGGKVEIKR
L12099 TR1.9	ELVM	RG	-W-	-	-S-	-	..LTFGGGKVDI
X63398 IGKV1-27*01	ELVM	-N-A-	-W-	-	-N-	-I-	..LTFGGGKVEI
X98976 126F09	DIQMTQSPSSLSASVGDRTVITCRAS	QGISNY	LAWYQKPKGAPKLLIY AAS	TLQSGVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QKNSAP	..LTFGGGKVEIKR
Z00001 IGKV1-5*01	1	30	40	60	80	90	110
AJ399874 T5	DIQMTQSPSTLSASVGDRTVITCRAS	QGISSSW	LAWYQKPKGAPKLLIY DAS	SLESQVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANSYS	..LTFGGGKVEIKR
Z00013 IGKV1-9*01	E-VL-HP-	-VTQ-	-R-	-K-	-N-	-F-	..YTFGGGKVEIKR
X98965 126A	DIQLTQSPFLSASVGDRTVITCRAS	QGISY	LAWYQKPKGAPKLLIY AAS	TLQSGVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANSYP	..LTFGGGKVEIKR
X98966 126C	V	T-D	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98968 126F	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98969 126G	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98970 126H	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98971 126I	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98975 126F08	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98977 126F010	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98978 126F015	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98979 126FP1	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98981 126FP6	V	T	F-K-S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98982 126FP7	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X98983 126FP8	V	T	S-N-S	S	N	N	-VYH-..LTFGGGKVEIKR
X17263 IGKV1D-12*01	DIQMTQSPSSASVGDRTVITCRAS	QGISSSW	LAWYQKPKGAPKLLIY AAS	SLQSGVP SRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOANSFP	..VLSFGGGTVEIKR
AJ399871 B7	-LL-T-V-	-R-TNL-	-Q-R-	-GY-	-R-	-T-	..VLSFGGGTVEIKR
X12691 IGRV2D-28*01	DIYMTQSPFLSVPPEASISCRSS	QSLHNGYNY	LDWYQKPKGAPKLLIY LGS	NRASQVP DRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOALQTP	..YTFGGGK
L12095 TR1.6	V	G	-	-	-	-	..YTFGGGK
L12097 TR1.8	EL	-F-	-	-	-	-	..YTFGGGKLE
L12114 TR1.37	EL	-F-	-	-	-	-	..YTFGGGKLE
X01668 IGRV3-11*01	EIVLTQSPATLSLSPGERATLSCRAS	QSVSSY	LAWYQKPKGAPKLLIY DAS	NRATGIP ARFSGG	SGTDFLTITSISSLPEDFAFYVC	QOASNWP	..LIVNFGGGTVEIKR
AF306380 T2.2	E	-I-N-	-	-	-	-NS-	..LIVNFGGGTVEIKR
AJ399872 T2	-TT-	S	-P-	-TA-	-R-	-R-	..SFGGGTQIVLIS
AJ399878 T10	-G-	-TV-	-	-	-	-	..LTFGGGKVEIKR
M23090 IGRV3-15*01	EIVMTQSPATLSLSPGERATLSCRAS	QSVSSN	LAWYQKPKGAPKLLIY GAS	TRATGIP ARFSGG	SGTDFLTITSISSLPEDFAFYVC	QOYNWP	..PFTFGGKVEIKR
X98990 131TF14-L-	..PFTFGGKVEIKR
X12686 IGRV3-20*01	EIVLTQSPGTLISLSPGERATLSCRAS	QSVSSSY	LAWYQKPKGAPKLLIY GAS	SRATGIP DRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOYSSP	..GAFGGGKVEIKR
AF306359 TF2.3	E	-TF-	-	-	-	-L-	..GAFGGGKVEIKR
AF306363 TF3.19	E	-	-	-	-	-N-	..RYTFGGGKVEIKR
AF306364 TF3.5	AE	-ANN-	S	-	-	-	..LTFGGGKVEIKR
AF306365 TF3.14	E	-T-	-L-	-	-	-	..LTFGGGKVEIKR
AF306379 T2.11	E	-F-	-	-	-	-F-N-	..RYTFGGGKVEIKR
AF306386 T3.15	E	-R-	-	-	-	-	..LTFGGGKVEIKR
X73854 2G4	E	-A-	-T-I-S-	-S-	-G-	-H-DM-	..PGITFGGKVEIKR
X73858 7F	E	-A-	-T-I-S-	-S-	-G-	-H-TFR-	..TFFGGGKVEIKR
Z00023 IGRV4-1*01	DIYMTQSPDLSLSPGERATLSCRAS	QSVLYSSNNKNY	LAWYQKPKGAPKLLIY WAS	TRESQVP DRFSGG	SGTDFLTITSISSLPEDFAFYVC	QOYFSTP	..LTFGGGKVEIKR
KM1	EL	-N-SRT-D-	-	-Q-	-	-D-	..LTFGGGKVEIKR
WR1.1223	..EL-	-P-I-	-	-	-	-G-	..FNS-

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 complementarity 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	110	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-S-O-FS	D.....	S-A.....	-A-R.....	-F.....
AJ399852 A13	-V.....	-T.....	-V.....	-V.....	-A.....	-A.....	YVFGTGLTVLIG
AJ399856 B1	-V.....	-D.....	-V.....	-D.....	-V.....	-V.....	DVFGTGKLEIKR
AJ399867 T7	-V.....	-D.....	-N.....	-T.....	-V.....	-V.....	RVFGGKLEIKR
AJ399868 T12	-V.....	-T.....	-F.....	-T.....	-V.....	-V.....	AFGGTGLVEIKR
AJ399869 T13	-S.....	-D.....	-D.....	-D.....	-RHV.....	-RHV.....	WVFGGKLEIKR
Z73654 IGLV1-44*01	QSVLTQPPS.ASGTQQRVTISCTGS	SSNIGSNY...	VHWYQQLPGTAPKLLIY	SNN.....	QRPSGVP.DRPSGSK.SGTSASLAISGLQAEDEADYYC	AAMDSSLSG	
AJ399847 A8	-P.....	-S.....	-C.....	-M.....	-D.....	-D.....	S.....
AJ399855 A17	[SV].....	-A.....	-C.....	-GKS.....	-D.....	-D.....	FVFGGKLEIKR
D87016 IGLV1-47*02	QSVLTQPPS.ASGTQQRVTISCTGS	SSNIGSNY...	VHWYQQLPGTAPKLLIY	RNN.....	QRPSGVP.DRPSGSK.SGTSASLAISGLQAEDEADYYC	AAMDSSLSG	
AJ238330 ICA5	-V.....	-T.....	-H.....	-G.....	-N.....	-N.....	VFGGKLEIKR
Z73661 IGLV1-51*01	QSVLTQPPS.VSAAQQRVTISCTGS	SSNIGNNY...	VSWYQQLPGTAPKLLIY	DNN.....	KRPSGIP.DRPSGSK.SGTSATLITGLQAEDEADYYC	GTWDDSSLSA	
AJ238329 ICA1	-R.....	S.....	-K.....	-F.....	-A.....	-E.....	CSKAAGNTY.....
AJ238331 ICB7	-V.....	-S.....	-L.....	-T.....	-E.....	-T.....	YVFGTGLVEIKR
AJ399840 A1	-V.....	-T.....	-L.....	-T.....	-E.....	-T.....	VFGGKLEIKR
AJ399841 A2	-V.....	-S.....	-F.....	-T.....	-E.....	-T.....	LVFGGKLEIKR
AJ399842 A3	-V.....	-M.....	-R.....	-Q.....	-Q.....	-Q.....	KVFGGKLEIKR
AJ399844 A5	-V.....	-T.....	-T.....	-T.....	-S.....	-S.....	LVFGGKLEIKR
AJ399846 A7	-V.....	-T.....	-T.....	-T.....	-S.....	-S.....	LVFGGKLEIKR
AJ399850 A11	-V.....	-AD.....	-F.....	-D.....	-E.....	-R.....	YVFGTGLTVLIG
AJ399851 A12	-V.....	-AD.....	-F.....	-D.....	-E.....	-R.....	VFGGKLEIKR
AJ399853 A15	-V.....	-T.....	-L.....	-I.....	-E.....	-R.....	YVFGTGLVEIKR
AJ399854 A16	-V.....	-S.....	-L.....	-I.....	-E.....	-R.....	YVFGTGLVEIKR
AJ399858 B4	-V.....	-T.....	-L.....	-I.....	-E.....	-R.....	YVFGTGLVEIKR
AJ399859 B5	-V.....	-N.....	-F.....	-D.....	-R.....	-F.....	VFGGKLEIKR
AJ399860 B6	-A-V-E.....	-AD.....	-F.....	-D.....	-E.....	-R.....	YVFGTGLTVLIG
AJ399861 B8	-V.....	-AD.....	-F.....	-D.....	-E.....	-R.....	VFGGKLEIKR
AJ399862 B9	-V.....	-G.....	-D.....	-S.....	-E.....	-R.....	VFGGKLEIKR
AJ399864 B11	-V.....	-G.....	-D.....	-S.....	-E.....	-R.....	VFGGKLEIKR
AJ399865 T1	-V.....	-N.....	-F.....	-D.....	-R.....	-F.....	VFGGKLEIKR
Z73664 IGLV2-14*01	QSAITQPAS.VSGSPQSVTISCTGT	SSDVGGNY...	VSWYQHPGKAPKLLIY	EVS.....	NRPSGVS.NRPSGSK.SGNTASLITGLQAEDEADYYC	SSYTSSTL	
AJ399843 A4	-V.....	-A.....	-R.....	-I.....	-Y.....	-G.....	T-A-P.....
AJ399863 B10	-V.....	-Q.....	-T.....	-S.....	-Y.....	-G.....	F.....
WR1.102	-E.....	-K.....	-V.....	-D.....	-R.....	-F.....	VFGGKLEIKR
X97462 IGLV2-8*01	QSAITQPAS.VSGSPQSVTISCTGT	SSDVGGNY...	VSWYQHPGKAPKLLIY	EVS.....	NRPSGVS.NRPSGSK.SGNTASLITGLQAEDEADYYC	SSYTSSTL	
AJ399866 T4	-FV.....	-T.....	-Y.....	-I.....	-N.....	-C.....	PI.....
X71966 IGLV3-21*01	SYVLTQPPS.VSVAQGTARITCGN	NIGSKS.....	VHWYQKPGQAPFLVIY	YDS.....	DRPSGIP.ERPSGSN.SGNTATLITSRVEAGDEADYYC	QVWDDSSDH	
U09085 TR1.41	EL-V.....	-A.....	-Q.....	-T.....	-S.....	-T.....	-R.....
X97474 IGLV3-25*01	SYELMQPPS.VSVSPQPTARITCGSD	ALPKQY.....	AYWYQKPGQAPFLVIY	KDS.....	ERPSGIP.ERPSGSS.SGTYTTLITSGVQAEDEADYYC	QASDSSGTY	
WR1.107	-V.....	-T.....	-H.....	-T.....	-V.....	-R.....	-Y.....
WR1.112	-V.....	-T.....	-H.....	-T.....	-V.....	-R.....	-Y.....
X14614 IGLV7-43*01	QTVVTOEPS.LTVSPGTVTLTICASS	TGAVTSGY...	PMWFOOKPGQAPRALIY	STS.....	NKHSWTP.ARPSGSL.LGKKAALTLGVOPEDEAEYIC	LVYVGGAO	
AJ399857 B3	-A.....	-P.....	-NI.....	-N.....	-R.....	-FR.....	VFGGKLEIKR

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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