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REVIEW

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

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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 editing 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

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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

(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.

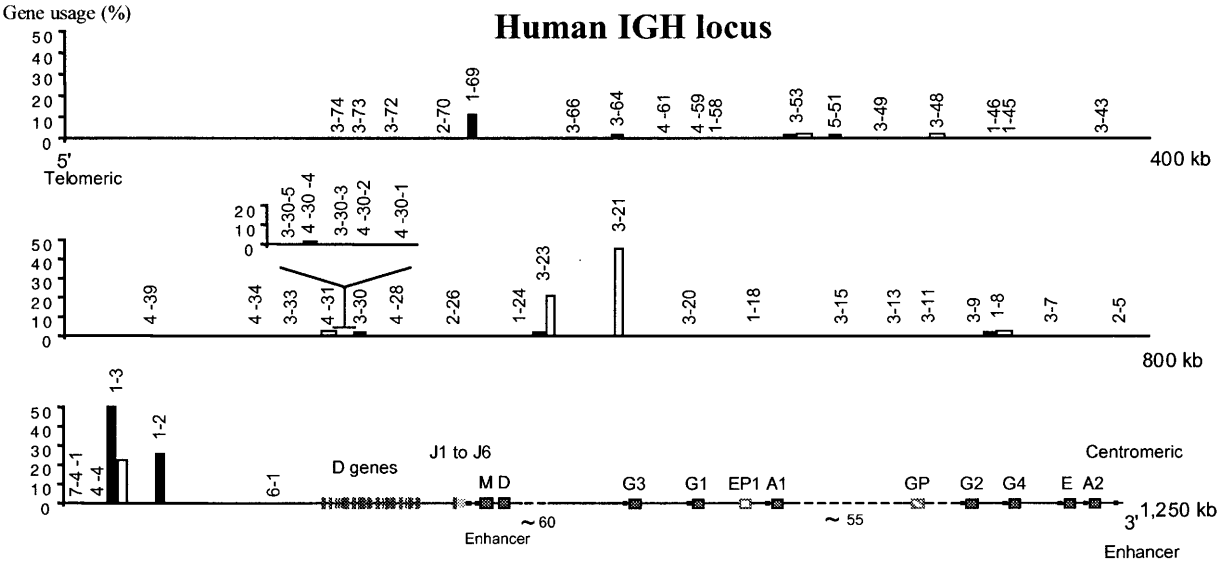
The D genes used by these aAb show a high diversity with a large number of genes in an inverted orientation of transcription (38%) (Table 1, consisting of parts a, b and c). Inverted D genes are rarely used by aAb, and this event seems to be a peculiarity of anti-TPO aAb. This observation suggests the possible involvement of particular mechanisms such as the use of D genes with irregular spacers (DIR elements) (Tuaillon and Capra 1998), preferential V-D rearrangements (Tuaillon and Capra 2000b), or modulation of terminal deoxynucleotidyltransferase activity (Tuaillon 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

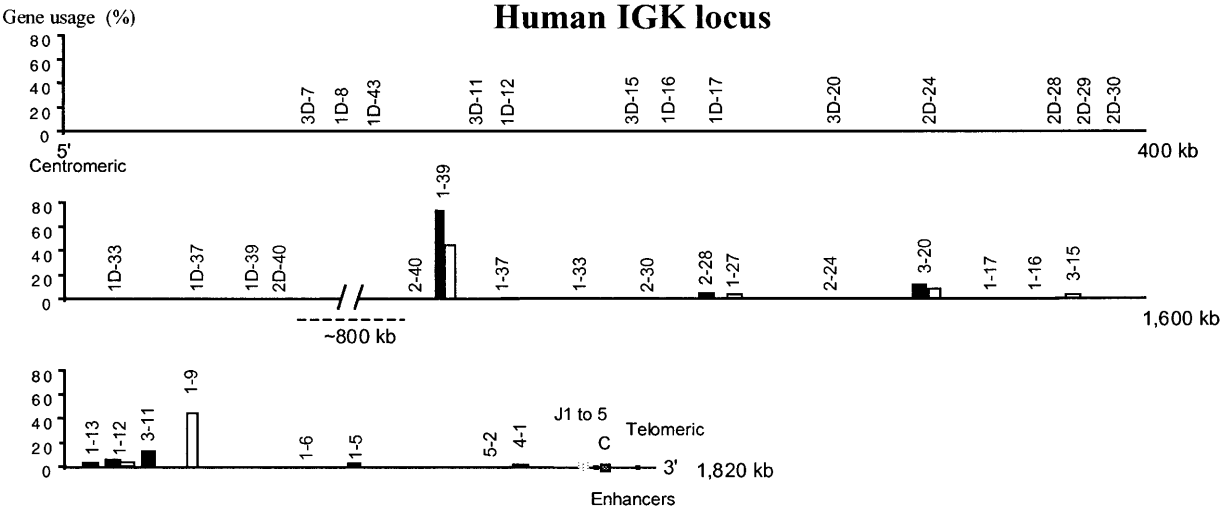
J distal *IGKV1* and *IGLV1* genes (Fig. 1) are preferentially rearranged in TPO-specific recombinant aAb (Tables 1 (consisting of parts a, b and c) and 2). Within

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 *IGKVI–12* and *IGKV1–39* could not be differentiated from their duplicated genes *IGKV1D–12* and *IGK1D–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


Human IGH locus
Gene usage (%)
5'
Telomeric
400 kb
800 kb
D genes
J1 to J6
M D
G3
G1
EP1 A1
GP
G2
G4
E A2
Centromeric
~ 60
~ 55
3'
1,250 kb
Enhancer
Enhancer

1b


Human IGK locus
Gene usage (%)
5'
Centromeric
400 kb
~800 kb
1,600 kb
J1 to 5
C
Telomeric
3'
1,820 kb
Enhancers

1c

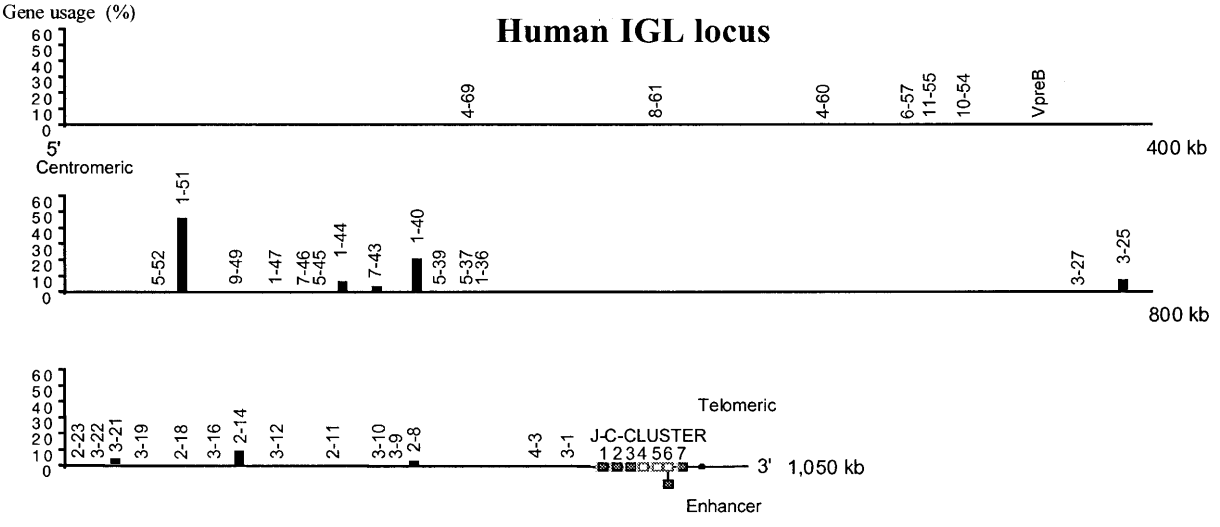

Human IGL locus
Gene usage (%)
5'
Centromeric
400 kb
800 kb
Telomeric
J-C-CLUSTER
1 2 3 4 5 6 7
3'
1,050 kb
Enhancer

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

Libraries[a]	Primer specificity	Clone	Heavy chain gene			Light chain gene[b]		Affinity[c] (nM)	TPO domain[d]
			IGHV	IGHD[g]	IGHJ	IGKV or IGLV	IGKJ or IGLJ		
Lambda phage libraries (λ >ZAP)[f]									
Fab from Graves'	γ1 and κ	SP1.2	IGHV1-2*02	ND	IGHJ6*02	IGKV1/1D-39*01	IGKJ2*01	0.08	IDR/A
thyroid B cells		SP1.4	IGHV1-2*02	ND	IGHJ6*02	IGKV1/1D-39*01	IGKJ3*01/4*01/5*01	0.22	IDR/A1
(Portolano et al., 1991; 1992)		SP1.5	IGHV1-2*02	ND	IGHJ6*02	IGKV1/1D-39*01	IGKJ3*01/4*01/5*01	0.06	IDR/A1
SP1-2 IGHV x different	γ1 and κ	SP1.12	id SP1.2	id SP1.2	id SP1.2	IGKV1/1D-39*01	IGKJ1*01		
IGKV (Roulette)		SP1.13	id SP1.2	id SP1.2	id SP1.2	IGKV1/1D-39*01	IGKJ2*01		
(Portolano et al., 1993b)		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		
		SP1.20	id SP1.2	id SP1.2	id SP1.2	IGKV1/1D-39*01	IGKJ1*01	0.09	IDR/A
SP1-2 IGKV x different	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
IGHV (Roulette)		SP1.7	IGHV1-2*02	ND	IGHJ6*02	id SP1.2	id SP1.2		IDR/A
(Portolano et al., 1993b)		SP1.9	IGHV1-2*02	ND	IGHJ6*02	id SP1.2	id SP1.2		IDR/A
Fab from Graves'	γ1 and κ	WR1.7	IGHV1-3*01	IGHD6-13*01	IGHJ4*02	IGKV1/1D-39*01d	IGKJ1*01d	0.2	IDR/A2
thyroid pan B cells		WR1. 9	IGHV1-3*01	IGHD6-13*01	IGHJ4*02	IGKV1/1D-39*01d	IGKJ1*01d		
(Chazenbalk et al., 1993)									
Fab from Graves'	γ4 and κ	WR4.2	IGHV1-2*02	IGHD2-2*01inv	IGH4[g]	IGKV1/1D-39*01	IGKJ2[g]		
thyroid pan B cells		WR4.3	IGHV1-2*02	IGHD2-2*01inv	IGH4[g]	IGKV1/1D-39*01	IGKJ2[g]		
(Chazenbalk et al., 1993)		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	0.31	IDR/A
		WR4.7	—[g]	—[g]	—[g]	IGKV1/1D-39*01	IGKJ1*01		
		WR4.8	IGHV1-2*02	IGHD2-2*01inv	IGH4[g]	IGKV1/1D-39*01	IGKJ2*01		
		WR4. 9	—[g]	—[g]	—[g]	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[g]	IGKJ2*01[g]		
		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'	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
thyroid pan B cells		TR1.5	IGHV3-53*01	IGHD6-6*01inv	IGHJ6*03	IGKV1/1D-39*01	IGKJ2*01		IDR/A:B
(Chazenbalk et al., 1993)		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[h]
		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	0.15	IDR/A
		TR1-13	IGHV1-3[g]	—[g]	IGHJ4[g]	IGKV1-13*02	IGKJ3*01		
Fab from Graves'	γ1 and κ	JA1.9	IGHV1-2*02	ND	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01		
thyroid pan B cells									
(Chazenbalk et al., 1993)									
Fab from Graves'	γ1 and κ/λ	KM1	IGHV3-30-3*01[g]	IGHD5-5*01[g]	IGHJ4[g]	IGKV4-1[g]	IGKJ4[g]	2.2	IDR/B
thyroid pan B cells		WR1.223	IGHV3-23*01[g]	IGHD3-9*01inv[g]	IGHJ3[g]	IGKV4-1[g]	IGKJ5[g]	0.81	IDR/B
(Jaume et al., 1997)									
Fab from Graves'	γ1 and κ	G(N) 1	IGVH1-2[g]	IGHD3-3/2-2[g]	IGHJ6[g]	IGKV3-11[g]	—[g]		
thyroid pan B cells		G(N) 2	IGHV1-3[g]	ND	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]		
(Guo et al., 1999)		G(N) 3	IGHV1-3[g]	ND	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]		
		G(N) 4	IGVH1-2[g]	IGHD3-3/2-2[g]	IGHJ6[g]	IGKV3-11[g]	—[g]		
		G(N) 5	IGHV1-3[g]	IGHD1-26inv/2-8inv[g]	IGHJ6[g]	IGKV1/1D-39*01[g]	—[g]		
		G(N) 6	IGHV1-3[g]	ND	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]		
		G(N) 7	IGHV1-3[g]	IGHD1-26inv/2-8inv[g]	IGHJ6[g]	IGKV1/1D-39*01[g]	—[g]		
		G(N) 9	IGHV1-3[g]	ND	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]		
		G(N) 17	IGVH1-2[g]	IGHD3-3/2-2[g]	IGHJ6[g]	IGKV3-11[g]	—[g]		
		G(N) 19	IGVH1-2[g]	IGHD3-3/2-2[g]	IGHJ6[g]	IGKV3-11[g]	—[g]		
		G(N) 22	IGVH1-2[g]	IGHD3-3/2-2[g]	IGHJ6[g]	IGKV3-11[g]	—[g]		
Filamentous phage libraries (phage display)[a]									
Fab from Graves'	γ1 and κ	TR1.21	IGHV1-2*02	IGHD3-16*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ4*01	0.35±0.11	
thyroid pan B cells		TR1.22	IGHV1-2*02	IGHD5-18*01inv/5-5*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ2*01		
(Portolano et al., 1993a)		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	0.57	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	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
thyroid pan B cells		7 F	IGHV4-31*01	IGHD3-10*01	IGHJ4*02	IGKV3-20*01	IGKJ1*01	80	not 2G4
(Hexham et al., 1994		10I	IGHV3-23*01	IGHD3-3*01/2	IGHJ6*02	IGKV1/1D-39*01	IGKJ3*01	9.3	not 2G4
Fab from Graves'	γ1 and λ	TR1.41	IGHV1-69*01	IGHD3-10*01	IGHJ3*02	IGLV3-21*01	IGLJ1*01	0.8	IDR/B
thyroid pan B cells		WR1.102	IGHV3-23[g]	IGHD3-22*01[g]	IGHJ4[g]	IGLV2-14[g]	IGLJ2*01[g]	2	IDR/B
(Prummel, 1994; Portolano, 1995)		WR1.107	IGHV1-2[g]	IGHD5-5*01[g]	IGHJ6[g]	IGLV3-25[g]	IGLJ2*01[g]	100	IDR/B
		WR1.112	IGHV4-30-4[g]	ND	IGHJ4[g]	IGLV3-25[g]	IGLJ2*01[g]	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		
Filamentous phage libraries (phage display)									
Fab from Hashimoto's γ1 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 1D-12*02	IGKJ4*01	0.2	
		126 F	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		
		126TO1	IGHV1-3*01	IGHD2-2*01inv/3inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ5*01	3.9	IDR/A
		126TO2	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ4*01	0.4-2.4	
		126TO3	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ4*01	0.4-2.4	
		126TO6	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*01	IGKV1/1D-39*01	IGKJ4*01	0.4-2.4	
		126TO8	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*02	IGKV1-9*01	IGKJ5*01	0.2-3.1	
		126TO9	IGHV3-21*01/2	IGHD2-21*01	IGHJ5*02	IGKV1-27*01	IGKJ4*01	0.094-10	
		126TO10	IGHV3-21*01/2	IGHD3-16*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01	0.094-10	
		126TO15	IGHV3-21*01/2	IGHD5-12*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01	0.094-10	
Fab from Hashimoto's γ1 and κ/λ lymph node pan B cells (McIntosh et al., 1997)		126TP1	IGHV3-21*01/2	IGHD3-16*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126TP5	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01		IDR/A
		126TP6	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126TP7	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*01/2	IGKV1-9*01	IGKJ4*01		
		126TP8	IGHV3-21*01/2	IGHD1-1*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126TP9	IGHV1-3*01	IGHD6-6*01inv/3-16*01 /3-10*01/2	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01		
		126TP10	IGHV3-21*01/2	IGHD3-16*01	IGHJ5*02	IGKV1-9*01	IGKJ4*01		
		126TP13	IGHV1-3*01	IGHD2-2*01inv/3inv	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01	2.8	
		126TP14	IGHV1-3*01	IGHD3-9*01inv	IGHJ6*02	IGKV1/1D-39*01	IGKJ4*01		
		126TP15	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 γ1 and κ thyroid pan B cells (Horimoto, 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; Pichurin et al., 2001)	γ1 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[g]	IGHD1-26inv/2-8inv[g]	IGHJ6[g]	IGKV1/1D-39*01[g]	—[g]		IDR
		DN 8	IGHV1-3[g]	IGHD1-26inv/2-8inv[g]	IGHJ6[g]	IGKV1/1D-39*01[g]	—[g]	0.15	IDR
		DN 14	IGHV1-3[g]	IGHD3-3/2-2[g]	IGHJ6[g]	IGKV3-11[g]	—[g]	0.26	IDR
		DN 15	IGHV1-3[g]	IGHD1-26inv/2-8inv[g]	IGHJ6[g]	IGKV1/1D-39*01[g]	—[g]		IDR
		DN 16	IGHV1-3[g]	IGHD1-26inv/2-8inv[g]	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]	0.12	IDR
		DN 20	IGHV1-3[g]	ND	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]		IDR
Fab from Graves' thyroid pan B cells (Guo et al., 1999)	γ1 and κ	N 2	IGHV1-3[g]	ND	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]		IDR
		N 5	IGHV1-3[g]	ND	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]		IDR
		N 6	IGHV1-3[g]	IGHD3-3/2-2[g]	IGHJ6[g]	IGKV3-11[g]	—[g]		IDR
		N 8	IGHV1-3[g]	ND	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]		IDR
		N 11	IGHV1-3[g]	ND	IGHJ4[g]	IGKV1/1D-39*01[g]	—[g]		IDR
		N 12	IGHV1-3[g]	ND	IGHJ4[g]	IGKV3-20[g]	—[g]		IDR
In-cell scFv from Grave thyroid CD19+ B cells (Chapal et al., 2000)	γ1 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)	γ1 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	VIII
		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	IGHJ3*01/2 /4*03	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 gen[a] IGHV	IGHD	IGHJ	Light chain gene IGKV or IGLV	IGKJ or IGLJ	Affinity (nM)	TPO domain
Filamentous phage libraries (phage display)									
scFv from Graves' thyroid pan B cells (Chapal et al., 2001)	γ1 and κ/λ	B1	IGHV1-3*01	IGHD5-24*01	IGHJ4*02	IGLV1-40*02	IGLJ3*02		
		B2	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ4*02	IGKV1/1D-39*01	IGKJ4*01	4.35	VI
		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	2.83	VI
		B5	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ1*01	IGLV1-51*01	IGLJ2*01/3*01	1.99	VI
		B6	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ1*01	IGLV1-51*01	IGLJ1*01	3.54	VI
		B7	IGHV1-3*01	IGHD2-21*01/2/3-10*01/2 /3-22*01	IGHJ4*02	IGKV1D-12*01	IGKJ5*01	2.17	VI/VIII
		B8	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ4*02	IGLV1-51*01	IGLJ3*02	0.99	VI
		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	12.3	VII
		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	IGHV1-3*01	IGHD4-4*01/4-11*01	IGHJ4*02	IGLV1-51*01	*ND*		
		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	1.28	VI/VIII
		T4	IGHV1-3*01	IGHD2-8*01inv/2inv /2-21*01inv/2inv	IGHJ4*02	IGLV2-8*01	IGLJ1*01		
		T5	IGHV1-8*01	IGHD3-3*02inv	IGHJ3*02	IGKV1-5*03	IGKJ2*01	0.77	VI/VIII
		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	4.50	VIII
		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	2.19	VI/VIII
		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	7.95	VIII
Fab from Graves' thyroid pan B cells (Pichurin et al., 2001)	γ1 and κ	TF2.3	IGHV1-69*03	IGHD3-10*01	IGHJ6*02	IGKV3-20*01	IGKJ2*01		non-IDR
		TF2.4	IGHV1-69*04	IGHD3-10*01	IGHJ6*02	IGKV1-12*01/2 /1D-12*02	IGKJ1*01	2.0	non-IDR
		TF2.6	IGHV1-69*02/4/6	IGHD3-10*01	IGHJ6*02	IGKV1/1D-39*01	IGKJ1*01		non-IDR
		TF2.10	IGHV1-69*04	IGHD3-10*01	IGHJ6*02	IGKV1/1D-39*01	IGKJ1*01	2.7	non-IDR
		TF3.5	IGHV1-69*04/6	IGHD3-10*01	IGHJ6*02	IGKV3-20*01	*ND*	1.2	non-IDR
		TF3.12	IGHV1-69*04/6	IGHD3-10*01	IGHJ6*02	IGKV1-39*01/02 /1/1D-39*01	IGKJ2*01		non-IDR
		TF3.14	IGHV1-69*04/6	IGHD3-10*01	IGHJ6*02	IGKV3-20*01	IGKJ4*01		non-IDR
		TF3.19	IGHV1-69*04/6	IGHD3-10*01	IGHJ6*02	IGKV3-20*01	IGKJ2*01		non-IDR
		T2.2	IGHV1-2*02	IGHD1-20*01inv/1-1*01inv /6-13*01/6-6*01	IGHJ6*02	IGKV3-11*01	IGKJ2*01	0.25	IDR
		T2.5	IGHV5-51*01	IGHD5-18*01/5-5*01	IGHJ6*02	IGKV1D-39*01	IGKJ4*01	0.4	IDR
		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	0.12	IDR
		T2.7	IGHV1-3*01	IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01		IDR
		T2.11	IGHV1-3*01	IGHD3-10*01	IGHJ4*02	IGKV3-20*01	IGKJ2*01	1.6	IDR
		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		IDR
		T3.3	IGHV1-3*01	IGHD2-21*02inv /2-15*01inv /2-2*01inv/2inv/3inv	IGHJ6*02	IGKV1/1D-39*01	IGKJ1*01	0.2	IDR
		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	0.22	IDR
		T3.5	IGHV1-3*01	IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01	0.12	IDR
		T3.7	IGHV1-3*01	IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ4*01		IDR
		T3.10	IGHV1-3*01	IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01		IDR
		T3.13	IGHV1-3*01	IGHD5-24*01inv	IGHJ4*02	IGKV1/1D-39*01	IGKJ1*01		IDR
		T3.15	IGHV1-3*01	IGHD3-10*01	IGHJ4*02	IGKV3-20*01	IGKJ4*01		IDR

[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 (ELISA inhibition, Biacore inhibition). IDR characterized according to Chazenbalk *et al.* (1993) and regions I-IX (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.
id: 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 IMGT/V-QUEST)

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

[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*×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 IMGT/V-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 *IGKV1D-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

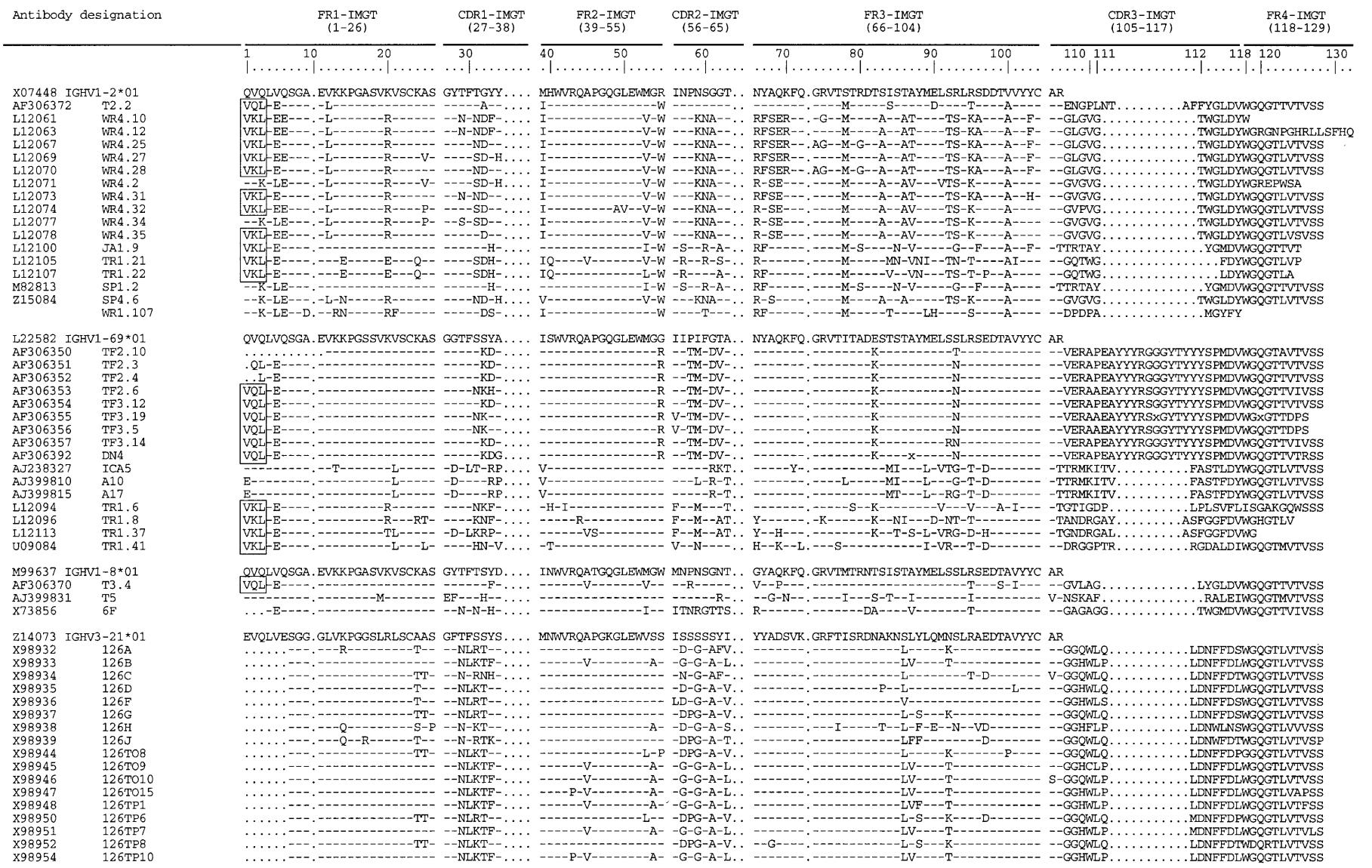
Whereas numerous somatic hypermutations are observed in TPO-specific heavy chains whatever the library origin (Table 3, consisting of parts a, b and c)), there is no or only limited amino acid replacement in most TPO-specific light chains, particularly those encoded by the J proximal *IGLV2–14*, *IGKV1–9*, *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 WR1.223, KMI, WR1.102, WR1.107, and WR1.112. 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)
		1 10 20	30	40 50	60	70 80 90 100	110 111 112 118	120 130
X62109 IGHV1-3*01		QVQLVQSGA.EVKKPGASVKVSCKAS	GYTFTSYA....	MHWVRQAPGQRLEWMGW	INAGNGNT..	KYSQKFQ.GRVTITRDTSASTAYMELSSLRSEDTAVYYC	AR	
AF306366	T2.11	VQL-E----.----------	--S-S--G....	V---------S------	-H--T---..	-------.D----I----------	--DPYSW	.....GGELDYWGQGTLVTVSS
AF306367	T2.6	VQL-E----.----------	-----H-S....	IN---------P-----	V-G---Y-..	R----L-.---N-------T----D----T-----I---	-KATLGA	.....LYGMDVWGQGTTVTVSS
AF306368	T3.2	VQL-E----.----------T-	-----H-S....	IN--------G-----Y	--G-----..	R-------.---NL--------V--D----------I---	-KATLGA	.....LYGMDVWGQGTTVTVSS
AF306369	T3.3	VQL-E----.-------A-R------	--MIAT-T....	---L-----H-P-----	---D--R-..	R---R--.---------I-N------N--T---------	-KVAAPAY	.....NFAVDVWGQGTTVTVSS
AF306371	T3.5	VQL-E----.----------	--S--I-P....	I---------N-----L	-HG-T---..	-------.----F----------NT--Y----M---	--SPYGD	.....LDYWGQGTLVTVSS
AF306374	T2.7	VQL-E----.Q----------	--S--I-P....	I----------L	-H---S--..	-------.----F----------Y----M---	--SPYSD	.....LDIWGQGTLVSVSS
AF306375	T3.10	VQL-E----.----------	--S--I-P....	----------	-H-------..	-------.----F----------Y----M---	--SPYGD	.....LDYWGQGTLVTVSS
AF306376	T3.13	VQL-E----.----------	--S--I-P....	I----------	-H-------..	-------.----F----------Y----M---	--SPYSD	.....LDYWGQGTLVTVSS
AF306377	T3.15	VQL-E----.----------	--A----G....	I----------I--	-SG-T---..	-------.A----------T---L----------	G-DPYGS	.....RGEMDYWGQGTLVTVSS
AF306378	T3.7	VQL-E----.x----------	--S--I-P....	I---------N-----L	-H--T---..	-------.----F----------Y----M---	--SPYSD	.....LDIWGQGTLVSVSS
AJ238326	ICA1	E---------.-M----------	--S-SI-N....	I---------G------	-H--T--R..	----V--.D----------S-------T---------	--DPNFG	.....DFDSWGQGTLVTVSS
AJ238328	ICB7	V---------.----------	--S-SI-N....	I---------G------	-H--T--R..	----V--.D----------S-------T---------	--DPNFG	.....DFDSWGQGTMVTVSS
AJ399801	A1	E---------.--R----------	--M-S--N....	I---------T------	-H--TS--..	-----L-.----F------N-------G-IP----I---	--DTYSD	.....FDYWGQGTLVTVSS
AJ399802	A2	E---------.-M-----T---------	--S-SV-N....	I---------G------	-H--T--R..	----M--.D----S------R-S-L-----T---------	--DPNYG	.....DFDYWGQGTLVTVSS
AJ399803	A3	E---------.----------	--A-A---....	I----------	-H--T--R..	----T--.D---L----TT----------K---------	--VPTGW	.....VAEFDSWGQGTLVTVSS
AJ399804	A4	E---------.---------RI--E--	--A-S--D....	I---------S------	-HG-T---..	-------.----F-------A-----T--T-----M-F-	--DTYRD	.....FDYWGQGTLVTVSS
AJ399805	A5	E---------.----------T-	--A-ST-I....	V----------	-HG-T---..	x--E---.-----------A----V-N---------F-	--DPNYG	.....DLDYWGQGTLVTVSS
AJ399806	A6	E---------.-M----------	--S-SI-N....	I---------G------	-H--T--R..	----V--.D----------S-------T---------	--DPNFG	.....DFDSWGQGTLVTVSS
AJ399807	A7	E---------.----------	--S-SI-N....	I---------G------	-H--T--R..	----V-E.D--S---------S-------T---------	--DPNFG	.....DFDSWGQGTLVTVSS
AJ399812	A13	E---------.-M----------	--S-SI-N....	I---------G------	-H--T--R..	----V--.D----------S-------T---------	--DPNFG	.....DFDSWGQGTLVTVSS
AJ399813	A14	L---------.-M----T---------	--S-SI-N....	I---------G------	-H--T--R..	----V--.D----------S-------T---------	--DPNFG	.....DFDSWGQGTLVTVSS
AJ399814	A16	L---------.----------	--S-SI-N....	I---------G------	-H--T--R..	----V--.D----------S-------T---------	--DPNFG	.....DFDSWGQGTMVTVSS
AJ399816	B1	E----E----.----------	--S-S--G....	I----------	--G-T-F-..	----N--.---SL--------S-L--NN-T----G--F-	--DPYNDY	.....AAELDYWGQGTLVTVSS
AJ399817	B2	E----E----.----------	--S-S--G....	I----------	--G-T-F-..	----N--.---SL--------S-L--NN-T----G--F-	--DPYNNY	.....AAELDYWGQGTLVTVSS
AJ399818	B3	E----E----.----------	--S-S--G....	I----------	--G-T-F-..	----N--.----L-----T----L--N-------GL---	--DPYNNN	.....AAELDYWGQGTLVTVSS
AJ399819	B4	--N-RE----.----------	--S-S--G....	I----------	--G-T-F-..	----N--.---SL--------S-L--NN-T----G--F-	--DPYNNY	.....AAELDYWGQGTLVTVSS
AJ399820	B5	E----E----.----------	--S-S--G....	I----------	--G-T-F-..	----N--.----L----------LD-N-------GL---	--DPYNNY	.....AAELDHWGQGTLVTVSS
AJ399821	B6	----------.----------	--S-S--G....	I----------	--G-T-F-..	----N--.----L----------LD-N-------GL---	--DPYNNY	.....AAELDHWGQGTLVTVSS
AJ399822	B7	----------.--RR-----R------	--S-ST-N....	I--L-------P--V-L	-HS-T---..	-SLE-LR.D----------T-V--------P--------	--EFYGD	.....FAYWGQGTLVTVSS
AJ399823	B8	E----E----.----------	--S-S--G....	I----------	--G-T-F-..	----N--.----L----------L--N-------G---	--DPYNNY	.....AAELDYWGQGTLVTVSS
AJ399824	B9	----------.--RR-----R------	--S-ST-N....	I--L-------P--V-L	-HS-T---..	-SLE-LR.D----------T-V--------P--------	--EFYGDF	.....AYWGQGTLVTVSS
AJ399825	B10	----L--A-.----------	--S-R--G....	I----------	--G-T-F-..	----I--.----L----A-----L--N-------GL---	--DPYNNY	.....AAEFDYWGQGTLVTVSS
AJ399827	T1	-----E----.----------	--S-S--G....	I----------	--G-T-F-..	----N--.---SL--------S-L--NN-T----G--F-	--DPYNNY	.....AAELDYWGQGTLVTVSS
AJ399828	T2	E---------.--RR------IT----	--S-ST-G....	I-----I----P--L--	-H--T-A-..	---REL-.--I-L------T-------R----------	--DLDPF	.....GGGMDVWGPGTLVTVSS
AJ399829	T3	E----E----.--RR-----RI-----	--S-ST-N....	I--L-------P--V-L	-HS-T---..	-SLE-LR.D----------T-V--------P--------	--EFYGD	.....FAYWGQGSLVTVSS
AJ399830	T4	-M--------.----------	--S--P-S....	I------A--S-----L	-Y--T-A-..	---DSW-.----F----------	--GLHPE	.....IDYWGQGTLVTVSS
AJ399832	T6	L---------.----------	--I--G-T....	I---------P-----	V-T-S-K-..	---E----.D----------I--------T---A-----	-GTGTTC	.....YMCLDYWGQGTLVTVSS
AJ399833	T7	L---------.--RR------IT----	--S-ST-G....	IQ---------P--L--	-H--T-G-..	----R----.--I---------N-V-LD-N--T---------	--DLDPF	.....GGGMDVWGQGTTVTVSS
AJ399834	T8	E----E----.--RR-----RI-----	--S-ST-N....	I--L-------P--V-L	-HS-T---..	-SLE-LR.D----------T-V--------P--------	--EFYGD	.....FAYWGQGSLVTVSS
AJ399835	T9	E---------.--RR-----R------	--S--T-T....	I--L-------P-----	-HS-----..	--LE-LR.D----------T----------P--------	--EFYGD	.....FAHWGQGTLVTVSS
AJ399837	T11	E---LE----.----------	-----G-T....	I---------P-----	V-T---K-..	---E---.D----------I--------T---A-----	-GTGTTC	.....YMCLDYWGQGTTVTVSS
AJ399838	T12	----L--A-.----------	--S-S--G....	I----------	--G-T-F-..	----N--.----L----------L--N-------GL---	--DPYNNY	.....AAELDYWGQGTLVTVSS
AJ399839	T13	E----E-A-.--RR------IT----	--S-ST-G....	IQ-M-------P--L--	-H--T-G-..	---R----.--I---------N-V-LD-N--T---------	--DLDPF	.....GGGMDVWGQGTTVTVSS
L12087	TR1.10	VKL-E----.----------	--I----H....	LY--------S------	--P-K----..	-------.----------L-MN----------	--VLGII	.....AADHWGPGTLVTVSSAS
L12098	TR1.9	VKL-E----.----------	--S----G....	L----------	-S--T---..	------R.----F-------T----G-----P--------	--DPYGGG	.....KSEFDYWGQGTLV
L12102	WR1.7	VKL-E----.-I--------L--E--	-D---N-M....	I----------	---D--KI..	R-------.----------S-------T---------	-SRGDSN	.....IWYLGYWGQGTL
L12103	WR1.9	VKL-E----.-I-------TL--E--	-D---N-M....	I----------	------KI..	R-------.----------S-------T---------	-SRGDSN	.....IWYLGYWGQGTLVT
L12109	TR1.23	VKL-E---P.---------TI-----	-F--NQ-T....	I----------	---A--K-..	R---N--.A-----T----N----D-TN-K-----L---	--SGVGSND	.....FHFALGYWGQGTLVT
X98940	126TO1	.....----.---R---T------T-	-FI-IN--....	I---------P-----	--NDS-R-..	-F-ENL-.----------L-----T-G--G----	-KRHDSGF	.....FSGMDVWGQGTMVTVSS
X98941	126TO2	.....----.-GRR------I---T-	-FIYIN--....	I---------P-----	----S-R-..	---E-L-.----------T-A-------	-KRQENAF	.....FSGMDVWGQGTMVTVSS
X98942	126TO3	.....----.---R------I---T-	-FIYIN--....	I---------P-----	----S-R-..	---E-L-.------P----------T-A--G-F--	--RQENAF	.....FSGMDVWGQGTMVTVSS
X98943	123TO6	.....----.---R------I---T-	-LIHIN--....	I---------P-----	----S-R-..	---E-LE.-----S----------I-T-A--T--D-	-KRQENTF	.....FSGMDVLGQGTMVTVSS
X98949	126TP5	.....----.---R------I---T-	-FI-IN--....	I---------P-----	----S-R-..	---E-L-.----------N---N--T-A-------	-KSQENVL	.....FSGMAVLAQGTLITVSS
X98953	126TP9	.....----.---R------I---T-	-FI-IN--....	I---------P-----	----S-R-..	-F-E-L-.----------V--------N-T-T--T----	TKRRENAF	.....FSGMDVWDQGTLVAVSS
X98955	126TP13	.....----.---R---T--G---T-	-FI-IN--....	I---------P-----	--NDS-R-..	---ENL-.----------L-----T-G-------	-KRHDSGF	.....FSGMDVWGQGTMVTVSS
X98956	126TP14	.....-A--.---R------I---T-	-FIYIN--....	I---------P-----	L---S-R-..	---E-L-.----------T-A-------	-KRQENAF	.....FSGIDVWDQGTMVTVSS
X98957	126TP15	.....-A--.---R------I---T-	-FIYIN--....	I---------P-----	L---S-R-..	---E-L-.----------T-A-------	-KRQENAF	.....FSGIDVWDQGTLVTVSS

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
AF306372 T2.2
L12061 WR4.10
L12063 WR4.12
L12067 WR4.25
L12069 WR4.27
L12070 WR4.28
L12071 WR4.2
L12073 WR4.31
L12074 WR4.32
L12077 WR4.34
L12078 WR4.35
L12100 JA1.9
L12105 TR1.21
L12107 TR1.22
M82813 SP1.2
Z15084 SP4.6
WR1.107
L22582 IGHV1-69*01
AF306350 TF2.10
AF306351 TF2.3
AF306352 TF2.4
AF306353 TF2.6
AF306354 TF3.12
AF306355 TF3.19
AF306356 TF3.5
AF306357 TF3.14
AF306392 DN4
AJ238327 ICA5
AJ399810 A10
AJ399815 A17
L12094 TR1.6
L12096 TR1.8
L12113 TR1.37
U09084 TR1.41
M99637 IGHV1-8*01
AF306370 T3.4
AJ399831 T5
X73856 6F
Z14073 IGHV3-21*01
X98932 126A
X98933 126B
X98934 126C
X98935 126D
X98936 126F
X98937 126G
X98938 126H
X98939 126J
X98944 126TO8
X98945 126TO9
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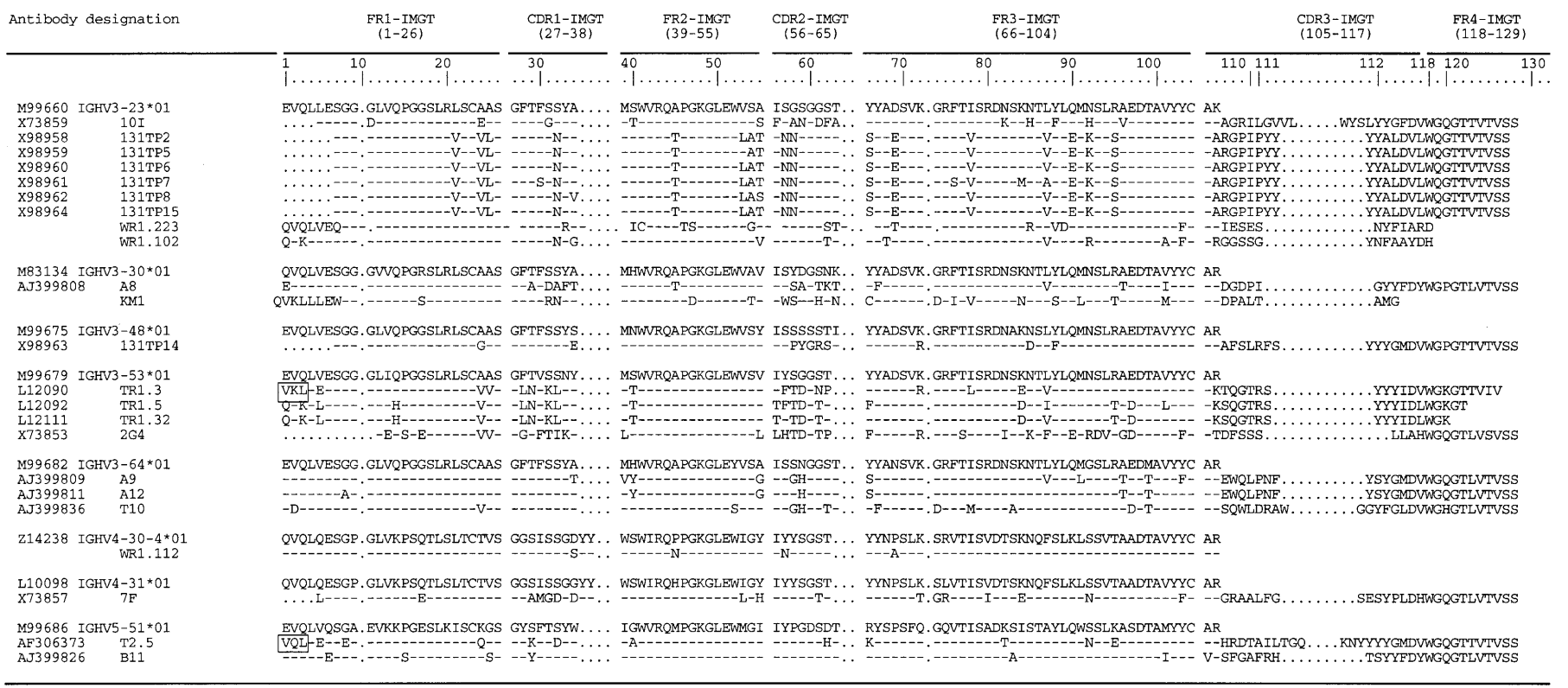
X98954 126TP10

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)
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X73859 10I
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X98961 131TP7
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WR1.223
WR1.102
M83134 IGHV3-30*01
AJ399808 A8
KM1
M99675 IGHV3-48*01
X98963 131TP14
M99679 IGHV3-53*01
L12090 TR1.3
L12092 TR1.5
L12111 TR1.32
X73853 2G4
M99682 IGHV3-64*01
AJ399809 A9
AJ399811 A12
AJ399836 T10
Z14238 IGHV4-30-4*01
WR1.112
L10098 IGHV4-31*01
X73857 7F
M99686 IGHV5-51*01
AF306373 T2.5
AJ399826 B11

Table 4a Amino acid sequences of human anti-TPO antibody *IGKV* 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 WR1.223 and KM1. 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)
	1 10 20	30	40 50	60	70 80 90 100	110	120
V01577 IGKV1-12*01	DIQMTQSPSSVSASVGDRVTITCRAS	QGISSW.....	LAWYQQKPGKAPKLLIY	AAS.....	SLQSGVP.SRFSGSG..SGTDFTLTISSLQPEDFATYYC	QQANSFP	
AF306360 TF2.4	ELV----------	------.....	----------	---.....	-------.-------..----------	-------....WTFGQGTKVEIKR	
AF306389 T3.4	ELV----------	-A-YT-.....	----------	---.....	-------.-------..---------N----------	--SY-T-....LTFGGGTKVEIKR	
X98967 126D	....----------	HR----.....	----------	---.....	N------.-------..----------	-------....LTFGGGTKVEIKR	
Z00006 IGKV1-13*02(F)	AIQLTQSPSSLSASVGDRVTITCRAS	QGISSA.....	LA*YQQKPGKAPKLLIY	DAS.....	SLESGVP.SRFSGSG..SGTDFTLTISSLQPEDFATYYC	QQFNNYP	
L12089 TR1.13	ELVM----------	----RG.....	--W----------	---.....	T------.-S-----..----------	----S--....LTFGPGTKVDI	
L12099 TR1.9	ELVM----------N-A----	-----.....	--W---------R----	---.....	N------.-------..----------I---	----S--....LTFGGGTKVEI	
X63398 IGKV1-27*01	DIQMTQSPSSLSASVGDRVTITCRAS	QGISNY.....	LAWYQQKPGKVPKLLIY	AAS.....	TLQSGVP.SRFSGSG..SGTDFTLTISSLQPEDVATYYC	QKYNSAP	
X98976 126TO9	....----------	------.....	----------	---.....	--E----.-------..-----N----N-----FG----	-QV-HY-....LTFGGGTKSEIK	
Z00001 IGKV1-5*01	DIQMTQSPSTLSASVGDRVTITCRAS	QSISSW.....	LAWYQQKPGKAPKLLIY	DAS.....	SLESGVP.SRFSGSG..SGTEFTLTISSLQPDDFATYYC	QQYNSYS	
AJ399874 T5	E-VL-HP-------I----------	--VTQ-.....	----------R----	K--.....	H-HD---.-------..---------N---------F--	---YT-P....YTFGQGTKLEIKR	
Z00013 IGKV1-9*01	DIQLTQSPSFLSASVGDRVTITCRAS	QGISSY.....	LAWYQQKPGKAPKLLIY	AAS.....	TLQSGVP.SRFSGSG..SGTEFTLTISSLQPEDFATYYC	QQLNSYP	
X98965 126A	....------V----------T	---D--.....	---------S--N---S	---.....	--H----.-------..-----N----N----------	--VYH--....LTFGGGTKVEIKR	
X98966 126C	....------V----------T	------.....	---------S--N---S	---.....	--H----.-------..-----N----N----------	--VYH--....LTFGGGTKVEIKR	
X98968 126F	....------V----------T	------.....	---------S--N---S	---.....	--H----.-------..-----N----N----------	--VYH--....LTFGGGTKVEIKR	
X98969 126G	....----------	------.....	-------S----N----	---.....	-------.-------..----------	--V----....VTFGQGTRLDIKR	
X98970 126H	....----------	------.....	----------	---.....	-------.-------..----------	-------....LTFGGGTKVEIKR	
X98971 126I	....------V----------T	---D--.....	---------S--N---S	---.....	--H----.-------..-----N----N----------	--VYH--....LTFGGGTKVEIK	
X98975 126TO8	....----------	------.....	-------S----N----	---.....	-------.-------..----------G----	--V----....VTFGQGTRLDIK	
X98977 126TO10	....------V-V----------T	---D--.....	---------S--N---S	V--.....	--H----.-------..-----N----N------G----	--VYH--....LTFGGGTKVEIK	
X98978 126TO15	....------G----------T	---D--.....	---------S--N---S	---.....	--H----.-------..-----N----N------G----	--VYH--....LTFGGGTKLEIK	
X98979 126TP1	....------V----------T	---D--.....	---------S--N---S	---.....	--H----.-------..-----N----N----------	--VYH--....LTFGGGTKVEIK	
X98981 126TP6	....------V-V----------T	---D--.....	---F-K---S--N---S	---.....	--H----.-------..-----N----N----------	--VYH--....LTFGGGTKVEIK	
X98982 126TP7	....------V----------T	----T-.....	---------S--N---S	---.....	--HT---.-------..-----N----N---D-------	--V-H--....LTFGGGTKVEIK	
X98983 126TP8	....------V----------T	---D--.....	---------S--N---S	---.....	--H----.-------..-----N----N------G----	--VYH--....LTFGGGTKVEIK	
X17263 IGKV1D-12*01	DIQMTQSPSSVSASVGDRVTITCRAS	QGISSW.....	LAWYQQKPGKAPKLLIY	AAS.....	SLQSGVP.SRFSGSG..SGTDFTLTISSLQPEDFATYYC	QQANSFP	
AJ399871 B7	--LL--T--V----------	-R-TNL.....	---------Q--R----	-GY.....	-------.---R---..---------T----------	--GY-S-...VLSFGGGTRLEIKR	
X12691 IGKV2D-28*01	DIVMTQSPLSLPVTPGEPASISCRSS	QSLLHSNGYNY.	LDWYLQKPGQSPQLLIY	LGS.....	NRASGVP.DRFSGSG..SGTDFTLKISRVEAEDVGVYYC	MQALQTP	
L12095 TR1.6	EL----------	-----G-----.	----------	---.....	-------.-------..----------	-----P-....YTFGQGTK	
L12097 TR1.8	EL------F----------	----------.	----------	-S-.....	S------.-------..----------	-----P-....YTFGQGTKLE	
L12114 TR1.37	EL----------	----------.	----------	---.....	T------.-------..----------	---R-I-....YTFGHFTK	
X01668 IGKV3-11*01	EIVLTQSPATLSLSPGERATLSCRAS	QSVSSY.....	LAWYQQKPGQAPRLLIY	DAS.....	NRATGIP.ARFSGSG..SGTDFTLTISSLEPEDFAVYYC	QQRSNWP	
AF306380 T2.2	..E----------	--I-N-.....	----------	---.....	S------.-------..----------	---NS---..LIYNFGQGTKLEIKR	
AJ399872 T2	-TT----------	-----S.....	----------P------	-TA.....	-------.-------..--R------T----------	---R---.....SFGGGTQLTVLS	
AJ399878 T10	--------G----------	-----TY.....	----------	---.....	-------.-------..----------	-------....LTFGGGTKLEIKR	
M23090 IGKV3-15*01	EIVMTQSPATLSVSPGERATLSCRAS	QSVSSN.....	LAWYQQKPGQAPRLLIY	GAS.....	TRATGIP.ARFSGSG..SGTEFTLTISSLQSEDFAVYYC	QQYNNWP	
X98990 131TP14	....----------	------.....	----------	---.....	-------.-------..----------G----	------L...PFTFGPGTKVDIK	
X12686 IGKV3-20*01	EIVLTQSPGTLSLSPGERATLSCRAS	QSVSSSY.....	LAWYQQKPGQAPRLLIY	GAS.....	SRATGIP.DRFSGSG..SGTDFTLTISRLEPEDFAVYYC	QQYGSSP	
AF306359 TF2.3	..E----------	-------.....	----------	---.....	-------.-------..----------	------L....GAFGQGTKLEIKR	
AF306363 TF3.19	..E----------	-----TF.....	----------	---.....	N------.-------..----------	----N--...RYTFGQGTKLEIKR	
AF306364 TF3.5	.AE----------	---ANN-.....	----------S---	---.....	-------.-------..----------	-------	
AF306365 TF3.14	..E----------T-	-----T-.....	-------L---------	---.....	-------.-------..----------	-------....LTFGGGTKVEIKR	
AF306379 T2.11	..E--------F----------	-------.....	----------	---.....	-------.H------..----------M-F-	--F-N--..RGYTFGQGTRLEIKR	
AF306386 T3.15	..E----------	-----R-.....	----------	---.....	-------.-------..----------	-------....LTFGGGTKVEIKR	
X73854 2G4	..E-----A------T-S----T-	-AATRA-.....	-T-I------S------	-S-.....	---A-V-.-------..----------G----------	-H-DM--..PGITFGHGTRLEIKRT	
X73858 7F	..E----------	-----NF.....	----------	---.....	T------.-------..----------	-H--TPR.....TFGQGTKVEIKRT	
Z00023 IGKV4-1*01	DIVMTQSPDSLAVSLGERATINCKSS	QSVLYSSNNKNY	LAWYQQKPGQPPKLLIY	WAS.....	TRESGVP.DRFSGSG..SGTDFTLTISSLQAEDVAVYYC	QQYYSTP	
KM1	EL----------	---N--SRT-D---	----------	-----.....	--Q----.-------..----------G----------	----D--	
WR1.223	..EL----------P-----	----I---------	----------	-----.....	-------.-------..----------G----------	---FNS-	

Table 4b

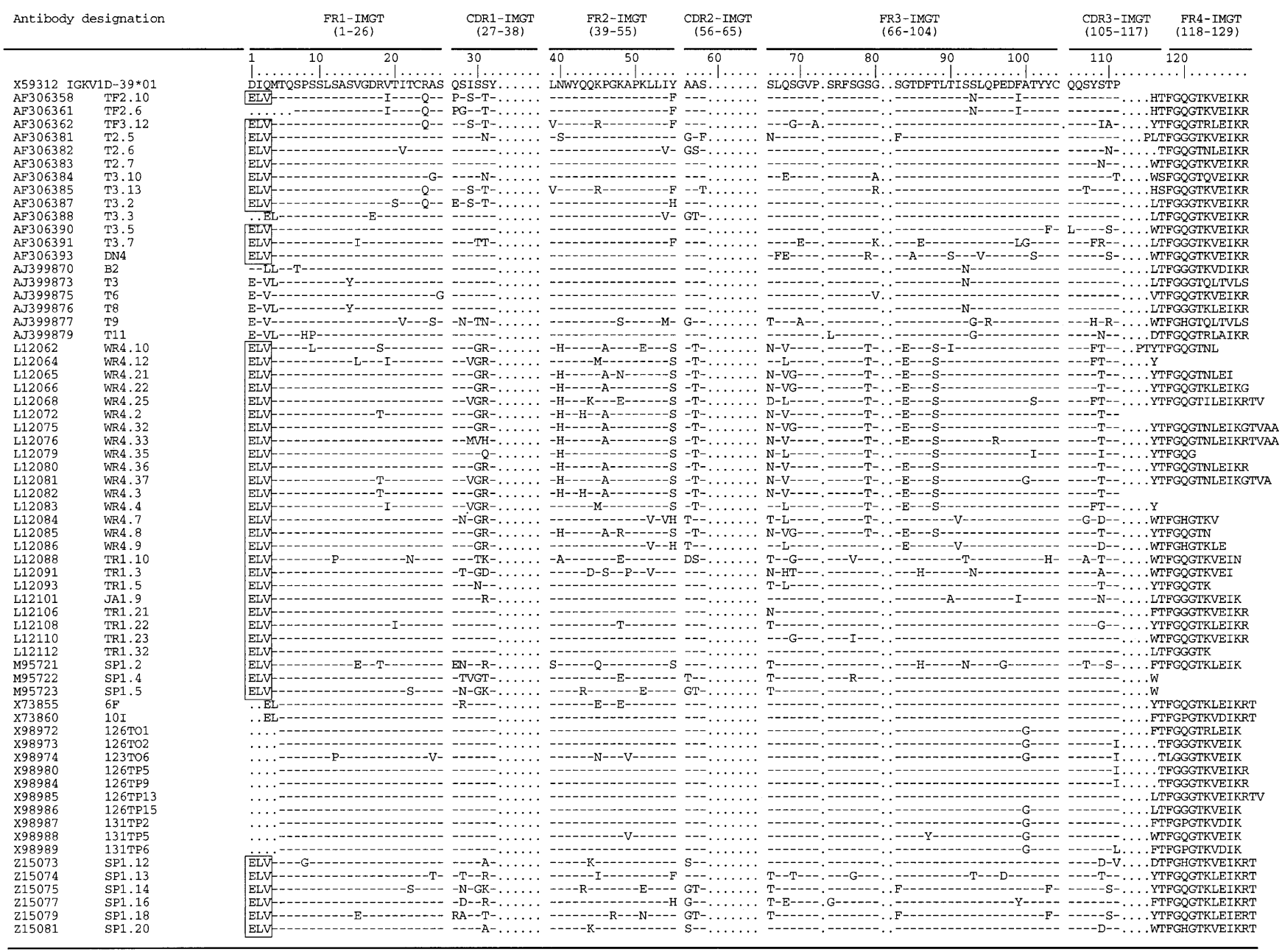
Antibody designation
FR1-IMGT (1-26)
CDR1-IMGT (27-38)
FR2-IMGT (39-55)
CDR2-IMGT (56-65)
FR3-IMGT (66-104)
CDR3-IMGT (105-117)
FR4-IMGT (118-129)
X59312 IGKV1D-39*01
AF306358 TF2.10
AF306361 TF2.6
AF306362 TF3.12
AF306381 T2.5
AF306382 T2.6
AF306383 T2.7
AF306384 T3.10
AF306385 T3.13
AF306387 T3.2
AF306388 T3.3
AF306390 T3.5
AF306391 T3.7
AF306393 DN4
AJ399870 B2
AJ399873 T3
AJ399875 T6
AJ399876 T8
AJ399877 T9
AJ399879 T11
L12062 WR4.10
L12064 WR4.12
L12065 WR4.21
L12066 WR4.22
L12068 WR4.25
L12072 WR4.2
L12075 WR4.32
L12076 WR4.33
L12079 WR4.35
L12080 WR4.36
L12081 WR4.37
L12082 WR4.3
L12083 WR4.4
L12084 WR4.7
L12085 WR4.8
L12086 WR4.9
L12088 TR1.10
L12091 TR1.3
L12093 TR1.5
L12101 JA1.9
L12106 TR1.21
L12108 TR1.22
L12110 TR1.23
L12112 TR1.32
M95721 SP1.2
M95722 SP1.4
M95723 SP1.5
X73855 6F
X73860 10I
X98972 126TO1
X98973 126TO2
X98974 123TO6
X98980 126TP5
X98984 126TP9
X98985 126TP13
X98986 126TP15
X98987 131TP2
X98988 131TP5
X98989 131TP6
Z15073 SP1.12
Z15074 SP1.13
Z15075 SP1.14
Z15077 SP1.16
Z15079 SP1.18
Z15081 SP1.20

Table 5 Amino acid sequences of human anti-TPO antibody *IGLV* 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 WR1.102, WR1.107, 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)
	1 10 20	30	40 50	60	70 80 90 100	110	120
M94116 IGLV1-40*01	QSVLTQPPS.VSGAPGQRVTISCTGS	SSNIGAGYD...	VHWYQQLPGTAPKLLIY	GNS.....	NRPSGVP.DRFSGSK..SGTSASLAITGLQAEDEADYYC	QSYDSSLSG	
AJ399845 A6	---V-----.----------	-----S--G...	----------	--F.....	-------.-------..----------	H---N----.	.DVFGTGTKLEIKR
AJ399848 A9	---------.--A--------T	T--------...	------F--S--Q--FS	-D-.....	S--A---.----A-R..----------P------F-	--F-R-K-P.	..LFGGGTKVTVLG
AJ399849 A10	---V-----.----------	---------...	----------	---.....	-------.-------..----------	--------.	.YVFGTGTKLTVLG
AJ399852 A13	---------.----------	---------...	-Q----------V---	---.....	-------.-------..----------	H---N----.	.DVFGTGTKLEIKR
AJ399856 B1	---V-----.----------	---------...	----------V------	---.....	-------.-------..----------	--------.	.RVFGGGTKLEIKR
AJ399867 T7	---------.--------A-V-----	-----D--...	----------	D--.....	-------.-------..----------	-------V.	..AFGGGTKLEIKR
AJ399868 T12	---V-----.---T-------	---------...	-N----------F---	--T.....	Y------.-------..----------	----N----.	.WVFGGGTKLEIKR
AJ399869 T13	-------S-.----------	---------...	----------	D-T.....	-------.-------..----------	-----RHWV.	...FGGGTKVTVVG
Z73654 IGLV1-44*01	QSVLTQPPS.ASGTPGQRVTISCSGS	SSNIGSNT....	VNWYQQLPGTAPKLLIY	SNN.....	QRPSGVP.DRFSGSK..SGTSASLAISGLQSEDEADYYC	AAWDDSLNG	
AJ399847 A8	-P-----S-.---------A------	-------P....	---C----------M-	GKS.....	-------.-------..-D----------	-S-----D-.	.PVFGGGTKLTVLG
AJ399855 A17	SYE------.----------	-------....	----------	---.....	-------.-------..----------	--------.	.CVFGTGTKVTVLG
D87016 IGLV1-47*02	QSVLTQPPS.ASGTPGQRVTISCSGS	SSNIGSNY....	VYWYQQLPGTAPKLLIY	RNN.....	QRPSGVP.DRFSGSK..SGTSASLAISGLRSEDEADYYC	AAWDDSLSG	
AJ238330 ICA5	---V-----.V--A---------T--	-----AG-D...	-H----------	G--.....	N------.-------..----------Q---------	------N-P.	..VFGGGTKLTVLG
Z73661 IGLV1-51*01	QSVLTQPPS.VSAAPGQKVTISCSGS	SSNIGNNY....	VSWYQQLPGTAPKLLIY	DNN.....	KRPSGIP.DRFSGSK..SGTSATLGITGLQTGDEADYYC	GTWDSSLSA	
AJ238329 ICA1	---------.-------R--------	--S---K-....	----------F	---.....	-------.----A--..----------E-------	CSKAAGNTY.	..VFGTGTKVDIKS
AJ238331 ICB7	---V-----.---------S------	---T----....	-----L----------	---.....	E------.-------..----------	--------.	.YVFGTGTKVDIKR
AJ399840 A1	---------.----------	--------....	----------	---.....	-------.-------..----------	--------.	.VVFGGGTKLTVLG
AJ399841 A2	---------.----------	-----S-F....	----------	---.....	-------.-------..----------	--------.	.LVFGGGTKVDIKR
AJ399842 A3	---------.-------M--------	--------....	-------R---------	---.....	Q------.-------..----------	--------.	.KVFGGGTKLTVLG
AJ399844 A5	-------S-.----------	T-------....	----------	---.....	-------.-------..----------	--------.	.LVFGGGTKVEIKR
AJ399846 A7	---V-----.----------	--------....	----------	---.....	------S.-------..----------	--------.	.LVFGGGTKVTVLG
AJ399850 A11	---------.----------	--------....	----------	G--.....	E------.-------..----------S-	-----R---.	.YVFGTGTKLTVLG
AJ399851 A12	---V-----.----------	--------....	----------	---.....	-------.-------..----------	--------G.	.VVFGGGTKVEIKR
AJ399853 A15	---------.----------	--------....	----------	G--.....	E------.-------..----------S-	-----R---.	.YVFGTGTKLEIKR
AJ399854 A16	---V-----.---------S------	---T----....	-----L----------	---.....	E------.-------..----------	--------.	.YVFGTGTKVDIKR
AJ399858 B4	---V-----.----------	--------....	----------	---.....	-------.-------..----------	--------.	.GVFGGGTKVEIKR
AJ399859 B5	---------.----------	N------F....	------F----------	--D.....	-------.------R..----------F--	----N----.	.VVFGGGTKLEIKR
AJ399860 B6	-A-V--E--.----------	----AD--....	----------	---.....	-------.-------..----------S--------	--------.	.GVFGTGTQLTVLG
AJ399861 B8	---------.----------	--------....	----------	---.....	-------.-------..----------	--------.	.GVFGGGTKLTVLG
AJ399862 B9	HA-------.----------	--------....	----------	-S-.....	-------.-------..----------	--------.	.GVFGGGTKLTVLG
AJ399864 B11	---------.----------G	--D----F....	----------	---.....	-------.-------..-------D-----P--------	-----G-NV.	..IFGGGTQLTVLS
AJ399865 T1	---V-----.----------	N------F....	------F----------	--D.....	-------.------R..----------F--	----N----.	.VVFGGGTRLEIKR
Z73664 IGLV2-14*01	QSALTQPAS.VSGSPGQSITISCTGT	SSDVGGYNY...	VSWYQQHPGKAPKLMIY	EVS.....	NRPSGVS.NRFSGSK..SGNTASLTISGLQAEDEADYYC	SSYTSSSTL	
AJ399843 A4	---------.----------A	-----T---...	------R-------I--	---.....	-------.-Y---G-.	----------	T--AP---F.	...FGGGTKLEIKR
AJ399863 B10	---------.----------	---------...	----------	D--.....	-------.-------..----------	--------R.	..VFGGGTKLTVLG
WR1.102	..E------.----------	-------K-...	----------V---	---.....	-------.-------..----------	------...	
X97462 IGLV2-8*01	QSALTQPPS.ASGSPGQSVTISCTGT	SSDVGGYNY...	VSWYQQHPGKAPKLMIY	EVS.....	KRPSGVP.DRFSGSK..SGNTASLTVSGLQAEDEADYYC	SSYAGSNNF	
AJ399866 T4	-PV------.----------	T---VD---...	------Y-------I--	--N.....	-------.N-------.---------C----------	------FI-.	..VFGSGTKLEIKR
X71966 IGLV3-21*01	SYVLTQPPS.VSVAPGKTARITCGGN	NIGSKS......	VHWYQQKPGQAPVLVIY	YDS.....	DRPSGIP.ERFSGSN..SGNTATLTISRVEAGDEADYYC	QVWDSSSDH	
U09085 TR1.41	EL-V----A.------Q--T-S---D	---T--.....	-------A--------S	--T.....	A------.-------..----------F--	----R--N-.	.YVFGTGTKVSVL
X97474 IGLV3-25*01	SYELMQPPS.VSVSPGQTARITCSGD	ALPKQY......	AYWYQQKPGQAPVLVIY	KDS.....	ERPSGIP.ERFSGSS..SGTTVTLTISGVQAEDEADYYC	QSADSSGTY	
WR1.107	..-------.----------	------.....	-H----------	--T.....	-------.--V-----.----------R---------	--------	
WR1.112	..-------.----------	------.....	-H----------	--T.....	-------.-------..----------R---------	-------Y	
X14614 IGLV7-43*01	QTVVTQEPS.LTVSPGGTVTLTCASS	TGAVTSGYY...	PNWFQQKPGQAPRALIY	STS.....	NKHSWTP.ARFSGSL..LGGKAALTLSGVQPEDEAEYYC	LLYYGGAQ	
AJ399857 B3	-A-------.----------	--P--NT--...	----------	N-N.....	------R.-------..----------	-VH---PR.	...VFGGGTQLTVLS

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 *IGKV1–39* 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 *IGHV1–69*, 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 *IGHV1–2* and *IGHV1–3*, as is also the case for a majority of the IDR aAb previously described (Table 1, consisting of parts a, b and c). Does *IGHV1–2* or *IGHV1–3* 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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