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Thyroid peroxidase autoantibody epitopic 'fingerprints' in juvenile Hashimoto's thyroiditis: evidence for conservation over time and in families

J. C. JAUME, C. L. BUREK*, W. H. HOFFMAN†, N. R. ROSE*, S. M. MCLACHLAN & B. RAPOPORT
*Thyroid Molecular Biology Unit, Veterans' Administration Medical Centre and the University of California, San Francisco, CA, *Department of Pathology and Department of Molecular Biology and Immunology, Johns Hopkins University, Baltimore, MD, and †Medical College of Georgia, Augusta, GA, USA*

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SUMMARY

In Hashimoto's thyroiditis, the humoral component is manifest by autoantibodies to thyroid peroxidase (TPO). Epitopic 'fingerprinting' of polyclonal serum TPO autoantibodies has been facilitated by the molecular cloning and expression as Fab of a repertoire of human TPO autoantibody genes. To investigate whether TPO autoantibody fingerprints are (i) stable over long periods of time (~15 years), and (ii) inherited, we studied a cohort of nine patients with juvenile Hashimoto's thyroiditis and 21 first degree relatives of four of these patients. Fingerprints were determined by competition between four selected Fab and serum autoantibodies for binding to ^{125}I-TPO. Regardless of titre, the TPO epitopic profile was stable in 10/12 individuals whose TPO autoantibody levels were sufficient for analysis on two or three occasions over 12–15 years. Although the TPO epitopic fingerprint profiles in two families raised the possibility of inheritance, overall the data from all four families did not reveal an obvious pattern of genetic control. In no family was the TPO epitopic fingerprint associated with HLA A, B or DR. In conclusion, TPO autoantibody epitopic fingerprints are frequently conserved over many years. Studies on additional families are necessary to establish whether or not the epitopic profiles of TPO autoantibodies are inherited.

Keywords autoantibody epitope fingerprint thyroiditis thyroid peroxidase

INTRODUCTION

Hashimoto's thyroiditis, the most common autoimmune disease affecting humans, is an example of organ-specific autoimmunity with a strong humoral component [1–3]. This humoral response is manifest by IgG class autoantibodies to thyroid peroxidase (TPO), previously known as the thyroid microsomal antigen (reviewed in [4]). In its native state, TPO is a large (≈210 kD), membrane-associated, homodimeric protein orientated towards the exterior surface of thyrocytes (reviewed in [4]). Mice immunized with purified human TPO generate antibodies which map to a large number of epitopes [5]. In contrast, spontaneously arising human autoantibodies to TPO recognize a more limited region on the molecule [5].

The molecular cloning and expression as Fab of a repertoire of human TPO autoantibody genes from thyroid-infiltrating plasma cells [6–11] has, for the first time, facilitated quantitative epitopic analysis of serum thyroid autoantibodies. Previous epitopic studies using murine MoAbs to TPO [5] and thyroglobulin [12,13] could not provide information on the relative proportions of autoantibodies to different epitopes in an individual serum. Competition studies with recombinant human Fab have revealed that polyclonal serum autoantibodies recognize an immunodominant region on TPO comprised of two, closely associated domains (A and B) [8]. Synthetic peptides or recombinant polypeptide fragments cannot be used to map epitopes within this region because the recombinant Fab [7,14], like most human TPO autoantibodies [5,15–17], preferentially recognize native (conformationally intact), not denatured antigen or antigenic fragments. However, using Fab which bind to four epitopes in the immunodominant region, it is possible to characterize an epitopic 'fingerprint' for TPO autoantibodies in an individual serum [18]. Furthermore, an intriguing observation is that the epitopic fingerprint of serum TPO autoantibodies is conserved, despite fluctuations in autoantibody levels, over 12 months in the post-partum period [19].

Conservation of TPO autoantibody fingerprints over a longer time interval in individuals, as well as among family members, would carry important implications for future genetic dissection of the inheritance of the humoral response in autoimmune thyroiditis. The present study, performed on a cohort of hypothyroid juveniles and some of their family members, demonstrates stability in the

Correspondence: Dr Juan Carlos Jaume MD, Thyroid Molecular Biology Unit (111T), Veterans' Administration Medical Centre, 4150 Clement Street, San Francisco, CA 94121, USA.

Table 1. Conservation over time of thyroid peroxidase (TPO) immunodominant region recognition by autoantibodies in the sera of hypothyroid juveniles with Hashimoto's thyroiditis

			TPO autoantibody titre*			TPO immunodominant region recognition (%)†		
Patient‡	Age§	0 years	2 years	15 years	0 years	2 years	15 years	
P1	(F)	10	200	300	450	100	91	81
S1	(F)	17	1000	400	600	82	85	92
J1	(F)	11	NA	300	400	NA	97	93
R1	(F)	10	600	1200	300	94	100	92
N1	(F)	8	400	400	180	40	43	60
W1	(F)	10	NA	600	30	NA	87	100
G1	(F)	15	2000	800	Un	93	94	–
H1	(F)	7	36	±	Un	87	–	–
B1	(M)	12	40	40	Un	56	32	–

* Reciprocal of dilution required to achieve ~15–20% ^{125}I-TPO binding.

† Inhibition by the pool of four TPO-specific human Fab that define the TPO immunodominant region (Subjects and Methods).

‡ Sex (F, female; M, male).

§ Age of patients at the initial (0 year) time point.

NA, Serum not available; Un, TPO autoantibodies undetectable; ±, TPO autoantibodies present, but levels insufficient for epitope characterization.

TPO autoantibody fingerprint in most patients and relatives over a period of 15 years, and suggests the possibility of genetic control of this phenotypic parameter.

SUBJECTS AND METHODS

Subjects

We studied the sera of nine children who presented with clinical hypothyroidism consequent to Hashimoto's thyroiditis, as well as sera from the families of four of these probands. These patients were identified in the Endocrinology Clinic, Children's Hospital of Michigan. Some of these sera were included in an earlier study of thyroid autoantibodies [20,21] performed before the development of TPO autoantibody assays and TPO autoantibody epitopic fingerprinting.

The initial serum samples were collected in 1977, followed by samples 2 years and 15 years later. Sera were stored in aliquots at –20°C. For two probands, sera were only available for the 2 year and 15 year time points. Sera were also available for 21 first degree relatives of four of the probands. For 20/21 relatives, serum samples were available at the two time points of widest separation, 2 and 15 years. For 13/21 relatives, serum samples were available at all three time intervals.

HLA-A and B were determined serologically [21]. HLA-DR alleles were analysed by the Immunogenetics Laboratory, Johns Hopkins University, in polymerase chain reaction (PCR)-amplified DNA using sequence-specific oligonucleotide probes [22].

TPO autoantibody binding

As antigen in the TPO autoantibody assay, secreted, recombinant human TPO [23] was labelled with ^{125}I to a specific activity of ≈50 μCi/μg protein by the iodogen method [24]. Duplicate aliquots of sera, diluted in assay buffer (0·1 M NaCl, 10 mM Tris–HCl pH 7·5, 0·1% Tween 20 and 0·5% bovine serum albumin (BSA)), were incubated for 1 h at room temperature with ^{125}I-TPO (20 000 ct/min) in a total volume of 200 μl. Protein A (Pansorbin; Calbiochem, La Jolla, CA; 50 μl) was added and the incubation continued for 30 min. After addition of 1 ml assay buffer, the mixture was vortexed, centrifuged for 25 min at 1400 ***g*** (4°C), supernatants were removed by aspiration and radioactivity in the pellets was counted. Non-specific ^{125}I-TPO binding in the presence of control serum without TPO antibodies was 2–5% of total ct/min added.

Preparation of soluble TPO-specific Fab protein

Fab were expressed as soluble proteins in XL1-Blue cells, as previously described [7,18]. In brief, transformed cells were grown (225 rev/min, 37°C) in SuperBroth medium with 100 μg/ml ampicillin, 20 mM $MgCl_2$, 1% glucose until an optical density (OD) of 0·2 was attained. Protein synthesis was then induced with 1 mM isopropyl-thio-galacto-pyranoside (Sigma Chemical Co., St Louis, MO) overnight at 27°C. The cells were then pelleted and resuspended in PBS pH 7·5, containing 2 μg/ml aprotinin, 1 μg/ml leupeptin, 1 μg/ml pepstatin, 0·1 mM phenylmethylsulfonyl fluoride (all from Sigma). The cells were sonicated and the supernatant was clarified by centrifugation at 30 000 ***g***. Fab in the supernatant were affinity-purified using a Protein G Sepharose column (Pharmacia, Piscataway, NJ), eluted with 0·1 M glycine pH 2·5 and neutralized immediately with 1 M Tris–HCl pH 7·5. Purified Fab were analysed by SDS–PAGE under reducing conditions [25].

Epitopic fingerprinting of polyclonal serum TPO autoantibodies

Epitopic fingerprinting of polyclonal serum TPO autoantibodies in an individual serum was performed as previously described [18]. Quantification of competition by the Fab was most accurately determined when sera bound 15–20% of the ^{125}I-TPO. Such dilution was necessary to attain maximal inhibition of TPO binding by the addition of an excess concentration of Fab. Therefore, as a first step, the assay described above was used to determine, for each individual serum, the dilution required to give ≈15% binding. These dilutions, which range between 1 : 12 and 1 : 2000, reflect the levels of TPO

Table 2. Conservation over time of thyroid peroxidase (TPO) immunodominant region recognition by autoantibodies in the sera of family members of hypothyroid juveniles with Hashimoto's thyroiditis

Family no.		Relation to proband	Age*	TPO autoantibody titre†			TPO immunodominant region recognition (%)‡		
				0 years	2 years	15 years	0 years	2 years	15 years
P	P3	Father		660	300	200	82	91	100
	P2	Mother		Un	Un	±	–	–	–
	P4	Sib (M)	12	±	±	12	–	–	100
	P5	Sib (F)	8	±	±	30	–	–	100
N	N3	Father		40	60	350	89	89	89
	N2	Mother		Un	Un	±	–	–	–
	N4	Sib (M)	16	Un	Un	Un	–	–	–
	N5	Sib (F)	15	Un	Un	±	–	–	–
	N6	Sib (F)	11	Un	Un	150	–	–	76
H	H2	Mother		40	20	30	94	96	97
	H3	Father		500	NA	NA	89	NA	NA
	H4	Sib (M)	14	NA	400	120	NA	61	69
	H5	Sib (F)	11	100	200	120	59	63	97
	H6	Sib (F)	5	NA	±	200	NA	–	96
	H7	Sib (F)	3	Un	Un	16	–	–	79
	H8	Sib (M)	1	NA	Un	Un	NA	–	–
	H9	Sib (F)	−3	NA	NA	±	NA	NA	–
W	W2	Mother		NA	40	60	NA	100	97
	W3	Father		NA	Un	±	NA	–	–
	W4	Sib (F)	13	NA	±	450	NA	–	99
	W5	Sib (F)	11	NA	Un	Un	–	–	–

* Age of offspring at the initial (0 year) time point.

† Reciprocal of dilution required to achieve $\sim 15-20\%$ ^{125}I-TPO binding.

‡ Inhibition by the pool of four TPO-specific human Fab that define the TPO immunodominant region (Subjects and Methods).

Un, TPO autoantibodies undetectable; ±, TPO autoantibodies present, but levels insufficient for epitope characterization; NA, serum not available.

autoantibodies in the study group. In some sera, although TPO autoantibodies were clearly detectable, their titre was insufficient to provide the necessary 15% tracer TPO binding necessary for epitopic fingerprinting.

Competition studies were subsequently performed by incubating appropriately diluted patient's serum with ^{125}I-TPO in the absence or presence of maximum concentrations (4×10^{-8} M) of TPO-specific Fab, either separately or as a pool. For greatest sensitivity of Fab inhibition, we used TPO within 7 days of isotopic labelling. Antigen–IgG complexes were precipitated with Protein A, as described above. Fab lack the CH2 domain of the Fc region and are not precipitated by Protein A. Non-specific ^{125}I-TPO binding (2–5% of total ct/min) by control serum without TPO antibodies was subtracted from the values obtained with patients' sera in calculating the percentage inhibition by the TPO-specific Fab.

Duplicate aliquots of each serum sample were analysed for competition between the four different Fab and the Fab pool. Sera from the same individual obtained at different time intervals, as well as sera from different individuals within a single family, were analysed in the same assay. In addition, epitopic fingerprints were determined (again in duplicate) in separate assays for 24 sera from different individuals. In every case, the same profile was obtained, indicating the reproducibility of the fingerprinting assay.

RESULTS

Conservation over time of TPO immunodominant region recognition

For seven of the nine juveniles with Hashimoto's thyroiditis and hypothyroidism, sera were available at three time intervals over a 15-year period (Table 1). Although serum was not available at the initial time point (0 years) for two individuals, the samples at the 2 year and 15 year time points were separated sufficiently widely to warrant study. TPO autoantibody levels, reflected by the titre giving ≈15% binding, tended to decline over the 13–15-year period of observation. Indeed, in three juveniles, serum TPO autoantibodies became undetectable at the 15 year time interval.

Regardless of the change in titre, there was remarkable consistency over the period of study in the proportion of autoantibodies in an individual serum whose binding to TPO was inhibited by the pool of recombinant Fab. In agreement with the previous observation that the recombinant Fab recognized immunodominant epitopes [8], the Fab inhibited the binding to TPO of serum autoantibodies in most (7/9) patients by more than 80% (Table 1). For two patients, at different time intervals, 32–60% of serum TPO autoantibodies were to the immunodominant region.

Sera were also obtained from the parents and siblings of four of the probands with juvenile Hashimoto's thyroiditis. A high proportion (all eight parents and 10/13 siblings) had TPO autoantibodies detectable on at least one occasion during the 15 year observation

period (Table 2). The range of TPO autoantibody titres in siblings (1 : 12–1 : 66, median 1 : 200) was lower than in probands (1 : 36–1 : 2000, median 1 : 600). In addition, autoantibody levels tended to rise (or become positive for the first time) at the 15 year observation point. However, as for the probands, TPO autoantibodies in the family members were consistently directed to the epitopic immunodominant region (Table 2).

Conservation over time of TPO autoantibody epitopic ' fingerprints' TPO epitopic fingerprints of polyclonal autoantibodies in the sera of probands and relatives were determined by means of competition studies using the individual Fab that define the immunodominant region. For simplicity, the nomenclature of the domains recognized by these Fab has been changed from previous publications [18,19]. The A domain is now subdivided into the A1 and A2 domains, based on recognition by Fab SP1.4 and WR1.7, respectively (Fig. 1a). Similarly, the subdivisions of the B domain, recognized by TR1.8 and TR1.9, are now annotated as B1 and B2, respectively.

The TPO epitopic fingerprints of polyclonal serum autoantibodies can be appreciated visually in a graphic representation (Fig. 1). Remarkably, for those individuals whose sera could be

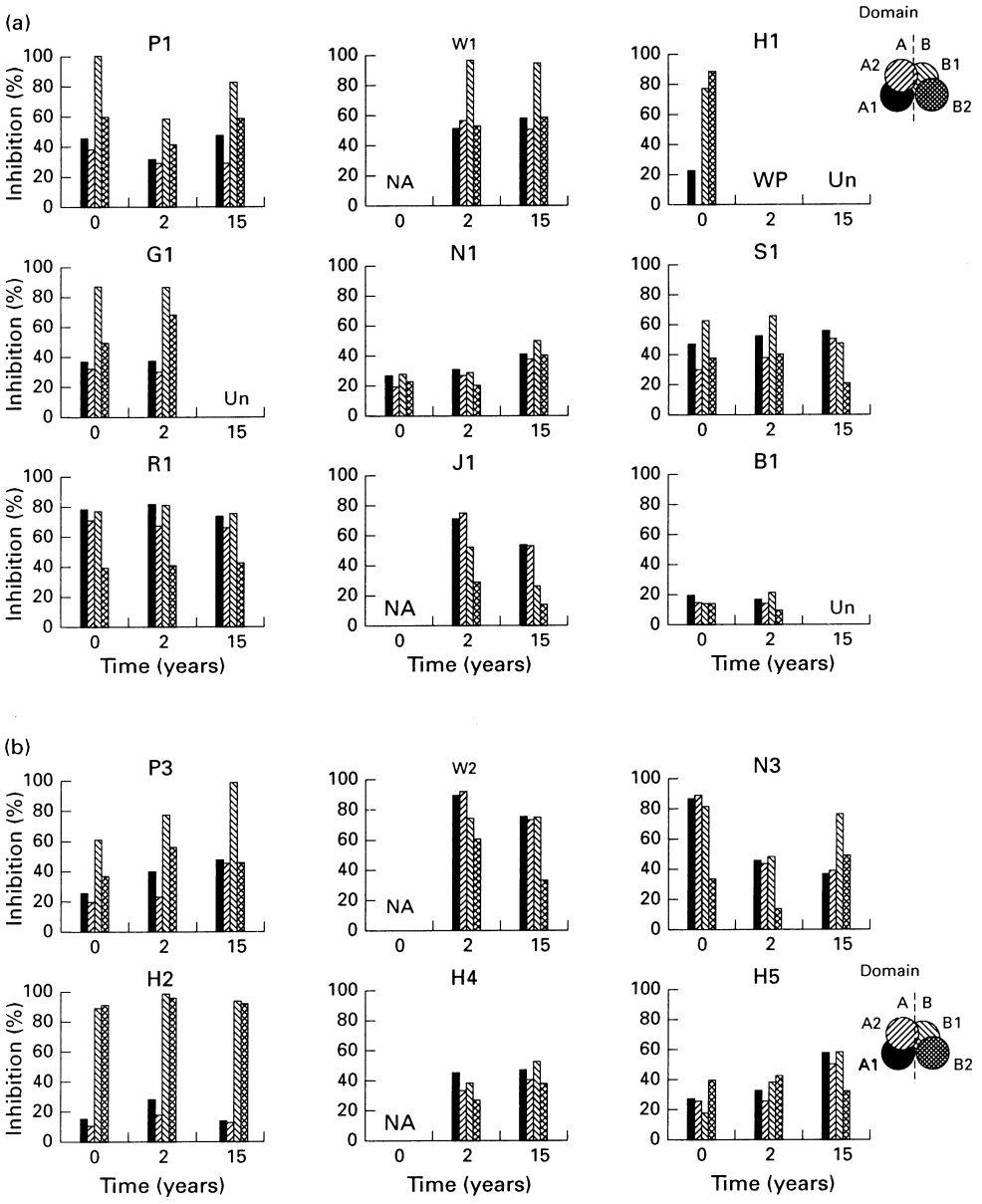

Fig. 1. Thyroid peroxidase (TPO) autoantibody epitopic fingerprints for (a) nine patients with juvenile Hashimoto's disease, and (b) six relatives of probands with juvenile Hashimoto's disease, characterized on two or three occasions over a period of up to 15 years. Four Fab to the A1, A2, B1 and B2 domains in the immunodominant region on TPO (see insets) were used to compete for serum autoantibody binding to ^{125}I-TPO. The percentage inhibition by each Fab is represented by a bar with shading corresponding to that in the inset. Sera collected in 1977 ('0 years') were not available for analysis (NA) for probands W1 and J1 and relatives W2 and H4. For sera collected 13 years later, TPO autoantibody levels were undetectable (Un) in three patients (H1, G1 and B1) or too low (WP, weak positive) to permit fingerprinting of the sera (H1, 2 years).

Table 3. Thyroid peroxidase (TPO) autoantibody epitopic profiles expressed as an A/B domain ratio

Individual	Patient or relative	Sex	TPO A/B domain ratio*		
			0 years	2 years	15 years
			Predominantly B domain		
H2	Mother (family H)	F	0·1	0·2	0·1
H1	Patient (proband)	F	0·4	–	–
W1	Patient (proband)	F	NA	0.5	0.4
P3	Father of P1	M	0·5	0·5	0·5
P1	Patient (proband)	F	0·5	0·5	0·6
G1	Patient	F	0·7	0·7	–
			A and B domains		
H5	Daughter (family H)	F	0·9	0·7	1·2
H4	Son (family H)	M	NA	1·2	1·0
N1	Patient (proband)	F	0·9	0·9	1·2
S1	Patient	F	0·8	0·9	1·6†
B1	Patient	M	1·0	1·2	–
R1	Patient	F	1·3	1·2	1·2
			Predominantly A domain		
W2	Mother (family W)	F	NA	1·4	1·4
J1	Patient	F	NA	1·8	>2·0
N3	Father (family N)	M	1·5	1·5	0·6†

* A/B domain ratio: $\frac{\text{Inhibition of } ^{125}\text{I-TPO binding by domain A1+A2 Fab}}{\text{Inhibition of } ^{125}\text{I-TPO binding by domain B1+B2 Fab}}$

† Shift in domain recognition over time.

–, TPO autoantibodies present, but levels insufficient for epitope characterization; NA, serum not available.

characterized, a particular epitopic profile was conserved over the period of observation. For five of the nine juvenile patients (P1, W1, R1, N1, J1), this conservation was observed over a 13–15 year time interval (Fig. 1a). In two patients (G1 and B1), TPO autoantibody epitopic fingerprints were conserved over 2 years; TPO autoantibodies were no longer detectable at the 15 year time point. The TPO epitopic profile in one patient (S1) was conserved for 2 years and then changed slightly over the next 13 years (increase in autoantibodies to the A2 subdomain). The fingerprint of patient H1 could only be determined at the initial time point.

Turning to the family members of four of the probands, six had TPO autoantibody titres sufficient for epitopic fingerprinting at the most widely separated time intervals (initial observation and 13–15 years later). In four of the six (P3, H2, W2 and H4), epitopic fingerprints were conserved (Fig. 1b). In the other two family members (N3 and H5), the conservation present after 2 years of observation was lost at the 15 year time point.

In addition to the visual representation of the TPO epitopic fingerprints, the same data could also be expressed mathematically as an A/B domain ratio (Table 3): (inhibition of ^{125}I-TPO binding by domain A1 + A2 Fab)/(inhibition of ^{125}I-TPO binding by domain B1 + B2 Fab). In general, the mathematical values agree with the visual perception (Figs 1 and 2) and confirm that, with a few exceptions, the TPO epitopic profile is conserved over a long period of time.

Relationship within a family of TPO epitopic fingerprints

The relative conservation over time of TPO epitopic fingerprints made it feasible to compare the TPO epitopic fingerprints among relatives, including the four probands with juvenile hypothyroidism. This analysis included family members from whom serum could be studied at only one time point and therefore comprises a larger number of individuals than those described in the time course studies (Fig. 1b). In individuals whose sera were studied at more than one time point, inhibition of TPO autoantibody binding by an individual Fab is calculated as the mean of the data at all time intervals.

Of the four families studied, a striking conservation of TPO epitopic fingerprints was observed in family P (Fig. 2a). Family H was remarkable in that TPO autoantibodies were present at sufficient titre for epitopic fingerprinting in both parents as well as in five of the seven siblings (Fig. 2b). Each parent had markedly different epitopic profiles, the father's autoantibodies being predominantly against TPO domain A, while the mother's autoantibodies were predominantly against TPO domain B. The proband, like her mother and one other female sibling (H6), had TPO autoantibodies primarily against the TPO B domain. Sibling H7 and her father had TPO autoantibodies with a bias towards the A domain. The two other siblings had intermediate phenotypes with approximately equal recognition of the A and B domains.

In two other families (N and W), with fewer individuals in whom fingerprinting could be performed, epitopic profiles varied among family members (Fig. 2c). In none of the four families was there an obvious relationship between HLA haplotypes and TPO epitopic domain recognition (Fig. 3).

DISCUSSION

Hypothyroidism in juveniles with Hashimoto's thyroiditis occurs less commonly than in adults. Previous studies showed that disease of early onset occurs in a population in which many parents and siblings have autoantibodies to thyroid microsomal antigen and/or

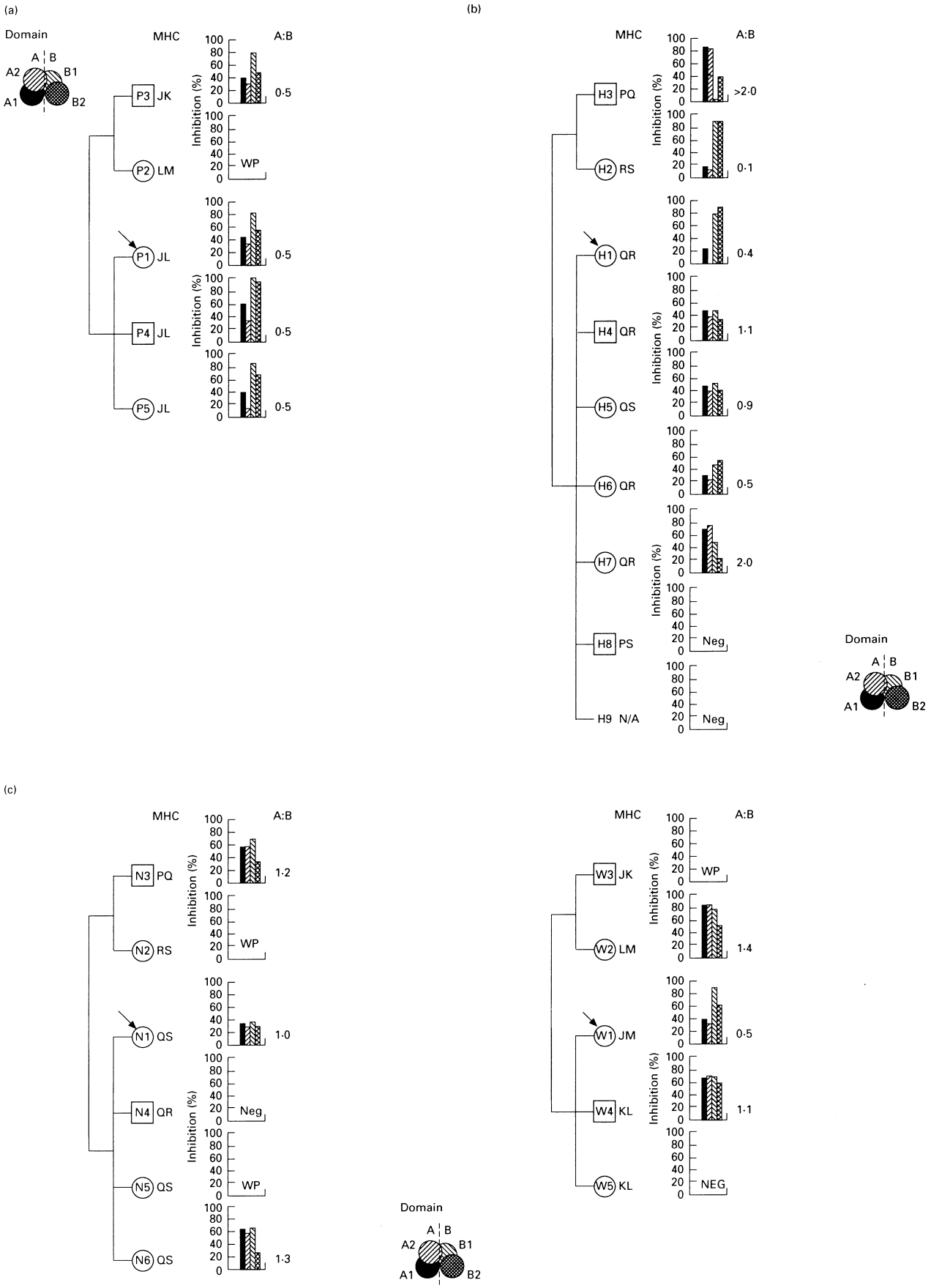
(a)
Domain
MHC
A:B
A
B
A2
B1
A1
B2
P3 JK
P2 LM
P1 JL
P4 JL
P5 JL
Inhibition (%)
WP
0·5
0·5
0·5
0·5
(b)
MHC
A:B
H3 PQ
H2 RS
H1 QR
H4 QR
H5 QS
H6 QR
H7 QR
H8 PS
H9 N/A
>2·0
0·1
0·4
1·1
0·9
0·5
2·0
Neg
Neg
Domain
(c)
N3 PQ
N2 RS
N1 QS
N4 QR
N5 QS
N6 QS
1·2
1·0
Neg
WP
1·3
W3 JK
W2 LM
W1 JM
W4 KL
W5 KL
1·4
0·5
1·1
NEG

Table 4. Prevalence of IgG class thyroid peroxidase (TPO) autoantibodies in different populations

	Prevalence of TPO autoantibodies*		
	% Female (*n*)	% Male (*n*)	Reference
Normal blood donors	18 (698)	10 (100)	[53]
Families unselected for autoimmune thyroid disease	28 (277)	9 (239)	[29]
Families with autoimmune thyroid disease	43 (117)	22 (77)	[28]
Juvenile Hashimoto's disease	77 (17)	75 (8)	This study

* Detected by serum binding of ^{125}I-TPO and precipitation with protein A [18,54].

thyroglobulin [20,26]. Using assays for TPO autoantibodies, the prevalence of these autoantibodies increases progressively in the following order: normal blood donors < families unselected for autoimmune thyroid disease < families with autoimmune thyroid disease ≪ families with a juvenile Hashimoto thyroiditis proband (Table 4). In the last group, both parents in all four families had TPO autoantibodies at one or another time point in the 15 year study period. The juvenile Hashimoto group is relatively small compared with the other three study groups. Nevertheless, the strikingly high proportion of TPO autoantibody-positive females as well as males in these families is consistent with the previous suggestion [20,26] that this form of disease occurs in a genetically highly predisposed population.

Although genetic factors clearly play a role in the pathogenesis of autoimmune thyroiditis, the mode of transmission of the ability to produce thyroid autoantibodies is unclear. Based on classical segregation analysis, the thyroid autoantibody response has been suggested to be an autosomal dominant trait [27–29] or multifactorial-polygenic trait [20,30–32]. More recently, using complex segregation analysis, one study could not distinguish between a single-gene and a multifactorial model [33], while data from an old order Amish population were consistent with autosomal dominant transmission of a major gene with a polygenic background [34].

Autoantibody epitopic fingerprint analysis could be an important phenotypic marker for investigating the complex genetic background to humoral autoimmunity, but only if such a fingerprint was relatively stable throughout the life of an individual. The present concept of epitope spreading, demonstrated for T cell [35] and some B cell [36,37] epitopes, would be incompatible with conservation of an autoantibody epitopic fingerprint. On the other hand, most studies of B cell epitope spreading have been demonstrated after artificial immunization. However, artificial immunization does not necessarily reflect the situation that occurs in spontaneous human autoimmune disease. For example, the epitopes recognized by human TPO autoantibodies are more limited than those of murine MoAbs generated by immunization [5].

The issue of autoantibody epitope conservation can only be answered in a longitudinal study. Such conservation was observed for serum TPO autoantibodies in a cohort of women with post-partum thyroiditis [19]. This observation is remarkable in view of the enhancement of the humoral immune response that occurs in the post-partum period. However, TPO epitopic profiles were only determined in these pregnant and post-partum women over a period of 1 year. Longitudinal studies on human autoantibodies to the immunodominant region on the acetylcholine receptor (characterized using murine MoAbs) over periods of 2–6 years support the concept of conservation of an epitopic profile [38,39]. The present study indicates remarkable conservation in the epitopic fingerprints of autoantibodies to the TPO immunodominant region in 10 of 12 individuals followed over a period of 13–15 years. These data provide the strongest evidence to date for restriction in the humoral response in spontaneous human autoimmunity. Conservation occurred despite marked changes in the overall titre of TPO autoantibodies. The stability of autoantibodies to other minor TPO epitopes, which typically constitute only ≈15% of serum autoantibodies, cannot be determined because of the lack of monoclonal Fab to these regions.

The occurrence and titres of thyroid autoantibodies in unusual families with juvenile hypothyroidism permit the assessment of TPO autoantibody epitopic fingerprints within a pedigree. The present study provides information on four of these families. Data from two families (P and H) are consistent with the possibility of genetic control. The TPO autoantibody epitopic fingerprint data from the other two families (N and W) are uninformative. Although genetic control in these families is not evident, this possibility cannot be excluded because two parents had levels of

Fig. 2. Thyroid peroxidase (TPO) autoantibody epitopic fingerprints in four families in which the proband (arrow) has juvenile Hashimoto's thyroiditis. The fingerprints are depicted graphically (shading as described in Fig. 1) and as the A:B domain ratio. (a) Family P: fingerprints for P1 and P3 are the mean values for sera collected at 0 years, 2 years and 15 years. MHC haplotypes: J – A26, B16, DR1; K – A9, B16, DR4*0404; L – A1, B17, DR4*0403; M – A1, B17, DR4*0402. (b) Family H: fingerprints for H3 and H5 are the mean values for sera collected at 0 years, 2 years and 15 years; in the case of H4, the fingerprint is the mean of two available samples (2 and 15 years). MHC haplotypes: P – A29, B40, DR4*0401; Q – A31, B7, DR15; R – A11, B18, DR13; S – A3, B44, DR7. (c) Families N and W. Fingerprints for N1 and N3 are the mean values for sera collected at 0 years, 2 years and 15 years. For the proband in family W (W1), the fingerprint is the mean of two available samples (2 and 15 years). MHC haplotypes for family N: P – A23, B44, DR4*0403; Q – A2, B18, DR11; R – A2, B8, DR3; S – A32, B38, DR4.01. MHC haplotypes for family W: J – A2, B44, DR4*0401; K – A24, B37, DR7; L – A2, B17, DR7; M – A1, B44, DR7.

TPO autoantibodies too low to permit fingerprinting. Study of additional families will be difficult because of the relative rarity of juvenile thyroiditis.

Families share environmental as well as genetic factors. In a comparison of monozygotic and dizygotic twin pairs, genetic rather than environmental factors were found to control levels of immunoglobulin and specific antibodies to tetanus toxoid or pneumococcal polysaccharide [40]. However, the nature of these genetic factors differs for different antibody responses. For example, inheritance of the ability to mount an autoantibody response to epidermal cadherin in healthy relatives of patients with pemphigus vulgaris is linked to HLA [41]. Similarly, HLA-DR and DQ genes control the autoimmune response to DNA topoisomerase I in systemic sclerosis [42]. In contrast, antibody responses to a major malarial antigen and some of its immunodominant epitopes, which were found to be more concordant between monozygotic than dizygotic twins, are not associated with HLA-DRB, DQA or DQB alleles [43].

Inheritance of autoimmune thyroid disease, although associated with MHC markers, is not linked to MHC markers [44,45]. Likewise, our data show no association between TPO autoantibody fingerprints and MHC, at least with respect to the class I and class II polymorphisms examined. Associations between autoimmune thyroid disease and candidate non-MHC markers, such as polymorphisms of the genes encoding immunoglobulins and T cell receptors, have yielded conflicting results (for example [46–49]). Potentially important is the association between CTLA4 alleles and one form of autoimmune thyroid disease, Graves' disease [50]. Polymorphisms in the CTLA4 gene, as well as other non-MHC genes including immunoglobulin heavy and light chain V region genes, and T cell receptor V genes, may play a role in the inheritance of TPO autoantibodies. Regardless of the markers studied, the most successful approach, used to identify genetic loci responsible for insulin-dependent diabetes mellitus [51,52], is likely to be positional cloning in sib-pairs concordant for the autoimmune response to TPO.

In conclusion, TPO autoantibody epitopic fingerprints are frequently conserved over many years. There is evidence, at least in some families, that autoantibodies with a particular TPO autoantibody epitopic profile are genetically controlled. These findings suggest that focusing on a particular TPO autoantibody response, for example antibodies directed against the B region of the immunodominant domain, may facilitate dissection of the genes responsible for the autoimmune response to TPO in humans.

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