Clin. exp. Immunol. (1989) 77, 21-24

Thyroglobulin antibodies in Graves' disease are associated with T-cell receptor beta chain and major histocompatibility complex loci

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(Accepted for publication 24 April 1989)

SUMMARY

We have investigated the T-cell antigen receptor constant beta and alpha chain genes (TCR-Cβ, -Cα) and the immunoglobulin (Ig) heavy chain switch regions of patients with Graves' disease (GD) using restriction fragment length polymorphism (RFLP) analysis. No significant associations were found with RFLPs of either the TCR-Cβ, -Cα or Ig heavy chain switch region loci and GD. However, a significant association was found between the presence of anti-thyroglobulin (anti-Tg) antibodies in the serum of patients and the 10·0; 9·2 kb TCR-Cβ genotype ($P < 0{\cdot}02$). Also, those patients with anti-Tg antibodies had an increased frequency of HLA-DR3 ($P < 0{\cdot}025$). These results suggest that genes residing in the TCR chain and major histocompatibility complex loci may be important in determining the immune response to thyroglobulin but not to the disease itself.

Keywords T-cell receptor major histocompatibility complex restriction fragment length polymorphism Graves' disease thyroglobulin

INTRODUCTION

Graves' disease is characterized immunologically by the presence of autoantibodies against the thyrotrophin (TSH) receptor, thyroid microsomal peroxidase and thyroglobulin (Tg). Lymphocytic infiltration of the thyroid gland is also present (Burman & Baker, 1985). There is ample evidence confirming the role of genetic factors in the predisposition to the disease. Most patients are female, they often possess HLA-DR3 and in certain populations particular combinations of immunoglobulin (Ig) constant heavy chain allotypes (Gm) (Farid & Bear, 1981). Until recently, investigation of the immunogenetic background of Graves' disease has been confined to serological studies (HLA and Gm). The recent cloning of the genes encoding these and other proteins important in the immune response allows the immunogenetic background to be dissected at the molecular level. A restriction fragment length polymorphism (RFLP) of the T-cell receptor constant beta chain (TCR-Cβ) gene has been shown to be associated with Graves' disease in a patient population from Newfoundland (Demaine *et al.*, 1987a). In contrast, a more recent study failed to find any significant association of the TCR-Cβ genes with Graves' disease in a group of caucasoid patients from the United Kingdom (Weetman *et al.*, 1988). A significant difference in the frequency of a TCR-Vα chain genotype has been described between autoimmune hypothyroidism and Graves' disease (Weetman *et al.*, 1987).

The role of TCR genes in contributing to the pathogenesis of Graves' disease is still unclear. The conflicting results of previous reports may reflect differences in the genetic background of the populations being studied, selection of the control or patient populations, or the autoantibody status of the patients. To try and answer these points we have investigated a well-defined group of British caucasoid patients with Graves' disease using DNA probes to the TCR-β and -α chain and Ig heavy chain loci. In addition, we have studied the frequency with which RFLPs are found in individual patients in association with the presence of autoantibodies at the time of presentation with untreated hyperthyroid Graves' disease.

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MATERIALS AND METHODS

Patients and controls

Sixty-five patients (49 women, 16 men) presenting to the Endocrine Clinic with clinical and biochemical evidence of hyperthyroidism were diagnosed as having Graves' disease when examination demonstrated physical signs compatible with this diagnosis and diffuse uptake was seen on a radioisotope scan of the thyroid gland. In addition, sera from all the patients had detectable autoantibody activity directed against at least one of the recognized autoantigens—thyroglobulin, thyroid microsome and the TSH receptor (Weetman *et al.*, 1986).

In addition, we studied 141 healthy blood donors with no known history of autoimmune disease, who served as control subjects.

DNA extraction and hybridization

Peripheral blood (25–35 ml) was taken from each subject into 5% EDTA and used to prepare high mol. wt DNA; 8–10 μg of DNA were digested with the appropriate restriction endonuclease, size-fractionated by electrophoresis in 0·6% agarose gels, and transferred to nylon membrane filters (Hybond-N, Amersham International, UK) using the method of Southern (Southern, 1975). Filters were hybridized for 16–20 h to a ^{32}P oligo-labelled probe (Feinberg & Vogelstein, 1983). Washes to remove non-specifically bound probe were carried out in 0·3 × SSC, 0·3% SDS for 45 min at 65°C. The filters were subsequently placed in Cronex daylight cassettes between Kodak XAR-5 film with two DuPont lighting plus intensifying screens at −70°C. Films were developed after 1–4 days.

DNA probes

Sμ probe. This was an 8-kb *Eco*RI genomic DNA fragment containing the Ig Sμ region which had been cloned in the vector pACYC184 (Flanagan & Rabbitts, 1982). A 2·1-kb fragment of Sμ was excised from the insert, using *Sst*I, and then separated from the vector in low melting-point agarose.

The Sμ probe hybridizes with Ig heavy chain switch regions flanking the Cμ and C α1 genes (Sμ and S α1, respectively) (Migone *et al.*, 1983). When used in conjunction with the restriction endonuclease *Sst*I the Sμ probe detects allelic fragments of 2·1 or 2·6 kb, giving genotypes of 2·1/2·1 kb; 2·6/2·6 kb or 2·1/2·6 kb homologous to the Sμ locus. At the Sα1 locus, allelic fragments of 7·4 or 6·9 kb are detected, giving rise to genotypes of 7·4/7·4 kb; 7·4/6·9 kb or 6·9/6·9 kb.

TCR-Cβ probe. The TCR-Cβ probe was obtained from a cDNA library derived from the Jurkat T-cell line. The cDNA homologous to the TCRβ chain gene was inserted into the *Pst*I restriction site of pBR322 (Yanagi *et al.*, 1984). The insert was excised from the plasmid using *Pst*I and was purified in low melting-point agarose. When used in conjunction with the restriction endonuclease *Bg*1II, allelic fragments of 9·2 or 10·0 kb are detected giving genotypes of 9·2/9·2 kb; 10·0/10·0 kb or 10·0/9·2 kb (Robinson & Kindt, 1985).

TCR-Cα, -Vα. This was obtained from a cDNA library of the Jurkat T cell line (Yanagi et al., 1985). The cDNA insert coding for the Cα and Vα sequences was excised from the plasmid pBR322 and purified in low melting-point agarose. When used in conjunction with the restriction endonuclease *Taq*I allelic fragments of 7·0 or 2·0 kb are detected giving genotypes of 7·0/7·0 kb; 2·0/2·0 kb or 7·0/2·0 kb corresponding to the TCR-Cα locus (So *et al.*, 1987). In addition, a 1·4-kb fragment may also be present which represents a polymorphism of the V α1 gene family. The Vα 1·4-kb fragment is either present or absent.

HLA typing

HLA typing was carried out using standard microcytotoxicity assay. The DR status of an individual was confirmed by genotyping using a DRβ probe (Bidwell *et al.*, 1987).

Autoantibody assay

Sera obtained from all patients at the time of presentation were stored at −20°C. All sera were analysed in the same assay for Tg and thyroid microsomal antibodies, using a conventional ELISA as previously described (Weetman *et al.*, 1986).

Statistical analysis

Statistical calculations were carried out using either 2 × 2 contingency tables or the χ^2 test; *P* values were corrected for the number of comparisons.

RESULTS

The frequencies of the TCR-Cβ chain genotypes in patients and controls are shown in Table 1. Patients were divided into two groups according to the presence or absence of anti-Tg antibodies prior to anti-thyroid treatment. It was not possible to separate the patients according to TSH receptor or thyroid microsomal antibody status, because >90% of the patients possessed these activities. Although there were no significant differences in the frequency of the TCR-Cβ genotypes between patients and controls, there were marked differences in the frequency of the TCR-Cβ genotypes between those patients with and without anti-Tg antibodies. Patients possessing anti-

Table 1. Frequency of TCR-Cβ chain genotypes and Tg antibody status

TCR-Cβ locus (kb)	Tg antibody status +		Tg antibody status −		Controls	
	n	%	*n*	%	*n*	%
10·0/9·2	22	62·9*	8	28·6	53	42·1
10·0	8	22·9	10	35·7	31	24·6
9·2	5	14·2	10	35·7	42	33·3

* Difference in frequency between patients with and without anti-Tg antibodies. $P < 0·02$ using a 2 × 2 contingency table with correction for the number of comparisons made.

Table 2. Frequency of TCR-Cα chain and Vα genotypes and Tg *n* antibody status

	Thyroglobulin antibody status +		Thyroglobulin antibody status −		Controls	
	n	%	*n*	%	*n*	%
TCR-Cα locus (kb)						
7·0/2·0	7	25·9	9	52·9	22	45·8
7·0	4	14·8	0	0·0	1	2·1
2·0	16	59·3	8	47·1	25	52·1
TCR-Vα locus						
Presence	16	59·3	12	70·6	33	68·8
Absence	11	40·7	5	29·4	15	31·2

Table 3. Frequency of Ig switch region genotypes and Tg antibody status

	Thyroglobulin antibody status					
	+		−		Controls	
	n	%	*n*	%	*n*	%
Sμ locus (kb)						
2·6; 2·1	15	51·7	13	59·1	79	54·1
2·6	10	34·5	6	27·3	29	19·9
2·1	3	10·3	2	9·1	36	24·7
2·1; 1·8	1	3·4	1	4·5	0	0·0
S α1 locus (kb)						
7·4; 6·9	17	56·7	8	40·0	68	47·6
7·4	10	33·3	9	45·0	53	37·1
6·9	3	10·0	3	15·0	22	15·4

Table 4. Frequency of HLA-DR3 and Tg antibody status

	HLA-DR3			
	+		−	
	n	%	*n*	%
Tg antibody				
+	22	68·8	10	31·2
−	8	36·4	14	63·6

Using a 2×2 χ^2 contingency table, $P < 0{\cdot}025$.

Tg antibodies had a significant increase in the frequency of the 10·0/9·2 kb TCR-Cβ genotype, compared with those without these antibodies (62·9% *versus* 28·6%; $P < 0{\cdot}02$, corrected).

No association was found between the TCR-Cα locus and Graves' disease itself, nor with the presence of anti-Tg antibodies (Table 2). Likewise, there was no association with the TCR-Vα RFLP which has been described previously to be associated with autoimmune hypothyroidism (Weetman *et al.*, 1988).

The frequencies of the Ig heavy chain switch region RFLPs (Sμ and S α1) and the presence of anti-Tg antibodies are shown in Table 3. No association was found with either switch region loci and the presence of anti-Tg antibodies or with Graves' disease itself.

Table 4 shows the frequency of HLA-DR3 with respect to anti-Tg antibody status. Those patients with anti-Tg antibodies had a significantly increased incidence of HLA-DR3, compared with those patients without this antibody (68·8% *versus* 36·4%, $P < 0{\cdot}025$).

DISCUSSION

The TCR-Cβ loci have been shown to be associated with type I diabetes and with membranous nephropathy, both of which are strongly associated with HLA-DR3 (Demaine *et al.*, 1987b; Hoover & Capra, 1987; Millward *et al.*, 1987). Recently, the TCR-Cβ loci have been shown to be associated with Graves' disease in caucasoid patients from Newfoundland but not with caucasoid patients from the United Kingdom (Demaine *et al.*, 1987a; Weetman *et al.*, 1988). These conflicting results may be a reflection of the genetic heterogeneity of the disease. For instance, it has been reported that HLA-DR3 has a higher incidence in Graves' disease patients from Newfoundland compared with those from the United Kingdom (Farid & Bear, 1981; Weetman *et al.*, 1986).

In this present study we found no association between the TCR-Cβ locus and Graves' disease. However, we did find a significant association between the TCR-Cβ 10·0/9·2 kb genotype and the presence of anti-Tg antibodies. The presence of these antibodies was also associated with HLA-DR3. At the present time there are few reports of 'immune response genes' in humans, mapping to any of the TCR loci. In the mouse, the immune response to pigeon cytochrome-c is restricted to a single TCR-Vα chain gene (Fink *et al.*, 1986; Sorger *et al.*, 1987). Seventeen T-cell lines specific for this antigen all possessed a TCR that utilized a member of the Vα 2B4 gene family and a limited number of Jα gene segments. In addition, one of three Vβ gene subgroups in conjunction with one of two Jβ gene segments was used in the expression of the TCR-β chain. The Vβ gene subgroup utilized correlated with the phenotype and fine specificity of the T-cell line.

Similarly, the immune response to hen egg lysozyme (HEL) is restricted to a Vα gene. A helper T cell hybridoma specific for HEL plus major histocompatibility complex (MHC) I-Aβ utilized the same TCR-Vα gene as T-cell lines obtained from mice immunized with HEL (Goverman *et al.*, 1985). A recent report of a study in humans suggests that the T cell response to the allogeneic MHC class II product DPw2 is restricted to the TCR-Vβ8·9 gene (Beall *et al.*, 1987). Finally, it has recently been demonstrated that T cells specific for myelin basic protein (MBP) obtained from mice with the autoimmune disease EAE use a Vβ gene of the Vβ8 subgroup. Further, immunization of these mice with anti-Vβ8 monoclonal antibody prevents the development of the disease (Acha-Orbea *et al.*, 1988; Urban *et al.*, 1988).

Our results suggest that the human TCR-β chain locus restricts the immune response to Tg. In this study only the TCR-Cβ chain genes were investigated, suggesting that the primary association lies within the Vβ, Dβ or Jβ genes. If the primary association were within the Vβ gene segments, then this would require the V and C genes to show linkage disequilibrium. At the present time the evidence would suggest that this is not the case, implying that the Dβ and Jβ gene segments may be important. Alternatively, the association may lie with a Vβ gene on the 3′ side of the Cβ genes such as the human equivalent of the murine Vβ 14 gene (Malissen *et al.*, 1986).

At present it is unclear whether the association between the TCR-β chain genes and anti-Tg antibodies is restricted to patients with Graves' disease. These antibodies are also found in other autoimmune diseases such as Hashimoto's thyroiditis,

type I diabetes, myasthenia gravis as well as in about 7% of healthy individuals. While reacting against the same molecule, it is possible that in different diseases and in healthy individuals these antibodies recognize different epitopes of Tg. Therefore, the TCR-β chain and MHC class II loci may be restricting the response to epitopes which are specific for Graves' disease. To clarify these points it will be necessary to investigate patients with other autoimmune disease who have anti-Tg antibodies. It would also be valuable to study a non-caucasoid group of patients with Graves' disease who have a different MHC association. An alternative approach would be to investigate a non-caucasoid population where anti-Tg antibodies are more prevalent than in the United Kingdom caucasoid population.

We have demonstrated that the TCR-β chain and MHC class II loci are associated with the presence of anti-Tg antibodies in patients with Graves' disease. It is not known whether these associations are specific for Graves' disease or for the immune response to Tg.

ACKNOWLEDGMENTS

The DNA probes used in this study were kindly provided by Drs T. H. Rabbitts and T. Mak.

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