Clin. exp. Immunol. (1990) **81**, 396–399

Lack of association of T cell receptor beta-chain constant region polymorphism with insulin-dependent diabetes mellitus in Finland

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(Accepted for publication 19 March 1990)

SUMMARY

Allelic polymorphism in the T cell receptor constant beta-chain gene region has been reported to be associated with autoimmune diseases, including insulin-dependent diabetes mellitus (IDDM). The present analysis of 164 children and adolescents with IDDM and 193 controls for *Bql*II polymorphism using a TcR-*Cβ* cDNA probe revealed two allelic restriction fragments with sizes of 10·5 kb (U) and 9·6 kb (L). No particular association was observed between the RFLP genotypes and IDDM (UU 27% *versus* 31%; UL 53% *versus* 52%; and LL 20% *versus* 17%, in diabetic subjects and controls, respectively), nor were any differences found between patients with various HLA risk antigens. The frequency of heterozygotes was 52% in 63 DR3-positive diabetic subjects and 53% in 73 DR3-negative ones. The results do not support any involvement of the TcR constant region genes in susceptibility to IDDM.

Keywords insulin-dependent diabetes mellitus T cell receptor beta-chain constant region polymorphism

INTRODUCTION

There are indications that an autoimmune process is involved in the pathogenesis of insulin-dependent diabetes mellitus (IDDM), the major susceptibility alleles for the disease being located in the HLA-DR/DQ region (Owerbach *et al.*, 1983; Cohen-Haguenauer *et al.*, 1985; Nerup, Mandrup-Poulsen & Molvig, 1987; Todd, Bell & McDevitt, 1987). The difference in concordance rate between HLA-identical siblings (16–25%) (Gorsuch *et al.*, 1982; Tarn *et al.*, 1988) *versus* monozygotic twins (30–50%) (Barnett *et al.*, 1981; Olmos *et al.*, 1988) nevertheless suggests that genes other than those for HLA may be involved in determining the risk of contracting the disease. Thus interest has been focused recently on the other genes coding for the key molecules in the immunological reactions. The activation of T cells is HLA-restricted, i.e. a T cell receptor (TcR) co-recognizes the foreign antigen and the self-class II HLA molecule. Accordingly, TcR and HLA class II molecules must contribute to the same immunological process. This enables a speculation that the presence of two different susceptibility genes (HLA and TcR) in a single individual may result in an abnormal immune response leading to IDDM. Differences in the immunoregulatory gene areas which undergo random somatic rearrangements could also explain the fact that at most only 50% of monozygotic twins are concordant for IDDM (Olmos *et al.*, 1988).

The TcR is composed of two polypeptide chains, an alpha chain and a beta chain or, alternatively, gamma and delta chains in a minority of T lymphocytes. Restriction fragment length polymorphism (RFLP) analysis has been used to detect polymorphic sites in these TcR genes. One such polymorphism revealed by the *Bgl*II enzyme is located 5′ to the second constant region gene (Robinson & Kindt, 1985). Heterozygosity for the bi-allelic restriction fragments has been associated with IDDM in Caucasian populations (Hoover & Capra, 1987; Millward *et al.*, 1987), whereas in Japanese patients the allele frequency of the smaller fragment was found to be increased (Ito *et al.*, 1988). Our results are contradictory to those mentioned above. We studied a large group of diabetic children and adolescents but could not find any association between IDDM and TcR-C*β* chain polymorphism in Finland, the country with the highest incidence of the disease in the world (Åkerblom & Reunanen, 1985).

SUBJECTS AND METHODS

Subjects

Blood samples were collected from 164 children and adolescents with IDDM on routine visits to the Diabetes Clinic at the Department of Paediatrics, University of Oulu. The controls were healthy blood donors and laboratory staff members from the city of Oulu.

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HLA-DR typing

Mononuclear cells were isolated from heparinized peripheral blood by Lymphoprep (Nyegard, Oslo, Norway) gradient centrifugation and B lymphocytes were further enriched by depletion of T cells with AET-treated sheep erythrocytes and of monocytes by plastic adherence. HLA-DR antigens were determined from B cells using the standard two-stage microlymphocytotoxicity method and commercial tissue typing trays (Biotest, Frankfurt, FRG).

The probe

The TcR-*Cβ* chain-specific cDNA probe was kindly provided by Dr T. Mak, Ontario Cancer Institute, Toronto, Canada.

RFLP analysis

The leucocytes were collected from EDTA blood samples by erythrocyte haemolysis (155 mM NH_4Cl, 10 mM $KHCO_3$, 0·1 mM EDTA, pH 7·4). The cell pellet of leucocytes and erythrocyte ghosts was suspended in TE buffer (10 mM Tris/HCl, 1 mM EDTA) and stored at −80°C. The cells were lysed at 37°C overnight (2 mM Tris, 0·4 mM EDTA, 2 mM NaCl, 1% SDS, 0·4% proteinase K) followed by phenol and chloroform extractions. DNA was precipitated from the aqueous phase with ethanol. The air-dried pellet was dissolved in an appropriate volume of TE buffer. Ten micrograms of DNA were digested with BamHI according to the manufacturer's specifications (Boehringer Mannheim, FRG) and electrophoresed in a 0·6% agarose gel at 40 V overnight. After an alkaline denaturation step, the DNA was transferred to nylon filters (Hybond-N, Amersham, UK) by the method of Southern (Southern, 1975), dried and u.v.-fixed for 3 min.

The purified TcR-*Cβ* probe was labelled with (alpha-^{32}P)dCTP by nick-translation to a specific activity of $3–5 \times 10^8$ ct/min per μg (Maniatis, Fritsch & Sambrook, 1982; Ausubell *et al.*, 1987). Hybridizations were performed at 42°C overnight in the presence of 50% formamide and 5% dextran sulphate. After two subsequent washings of 10 min at room temperature in 2 × SSC, 0·1% SDS and three washings of 20 min at 65°C in 0·1 × SSC, 0·1% SDS, the filters were exposed to an X-ray film for 2–3 days at −70°C with intensifying screens.

Statistical analysis

The statistical significances of differences between the groups were analysed by χ^2 test.

RESULTS

Restriction endonuclease *BglII* detects a bi-allelic polymorphism with bands at 9·6 kb (L) and 10·5 kb (U) (Fig. 1). Table 1 shows no differences between the diabetic and control subjects in the distribution of the TcR-*Cβ* genotypes or in the allele frequencies. The genotype frequencies were in Hardy–Weinberg equilibrium in both groups.

The diabetic subjects are grouped in Table 2 on the basis of their DR-antigen type, which again revealed no differences.

DISCUSSION

Several autoimmune diseases including IDDM (Hoover & Capra, 1987; Millward *et al.*, 1987; Ito *et al.*, 1988) have been studied for TcR-*Cβ* polymorphism and associations have been reported with Sjögren's syndrome (Freimark, Pickering & Fox, 1987), membraneous nephropathy (Demaine *et al.*, 1988) and Graves' disease (Demaine *et al.*, 1987); however, there have been controversial reports regarding different ethnic groups of subjects studied with Graves' disease (Weetman *et al.*, 1988; Demaine *et al.*, 1989). So far, no association has been found in the case of systemic lupus erythematosus (SLE) (Duncley, Gatenby & Serjeantson, 1988; Wong *et al.*, 1988) or myasthenia gravis (Oksenberg *et al.*, 1988). The present study on diabetic patients does not confirm the significance of genetic polymorphism in the constant region of the TcR beta chain as a marker determining the risk of IDDM. This is contradictory to previous

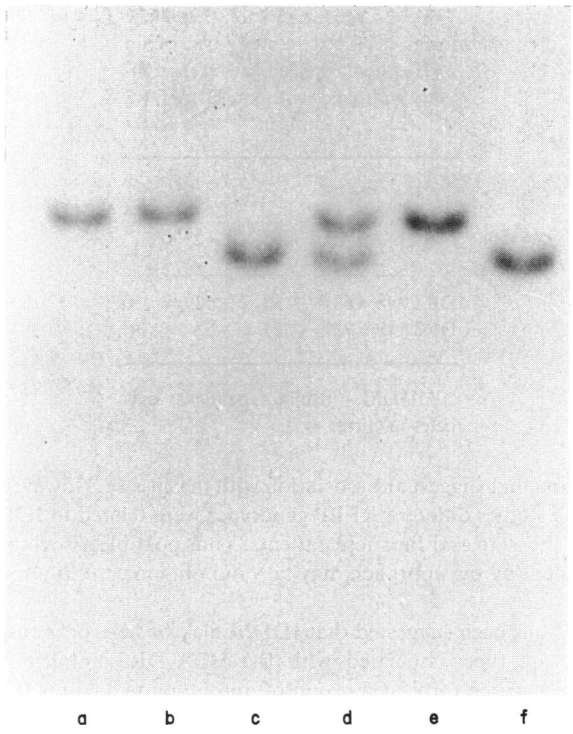

Fig. 1. The bi-allelic RFLP pattern detected by TcR-*Cβ* probe after *BglII* digestion. Upper restriction fragment is 10·5 kb (U allele) and the lower one is 9·6 kb (L allele). Lanes a, b and e show a homozygous UU genotype, lanes c and f LL genotype and lane d a heterozygous genotype UL.

Table 1. Frequencies of the allelic forms of T cell receptor beta-chain *BglII* polymorphism detected with the TcR-*Cβ*-cDNA probe

	Genotypes (%)		
	UU	UL	LL
IDDM ($n=164$)	27	53	20
Controls ($n=193$)	31	52	17

IDDM, insulin-dependent diabetes mellitus.

Table 2. Frequencies of the allelic forms of T cell receptor beta-chain *Bgl*II polymorphism in IDDM subjects with and without HLA-DR3 antigen

	Genotypes (%)		
	UU	UL	LL
DR3$^+$ (n=63)	30	52	18
DR3$^-$ (n=73)	27	53	19

IDDM, insulin-dependent diabetes mellitus.

reports that suggest an association with the disease. However, in those studies different RFLP genotypes were related to IDDM in Caucasian and Japanese patients. Thus possible associations detected by the approach may be valid only in certain populations.

It has been suggested that IDDM may be heterogenous, so that the type associated with the HLA-DR3 antigen may represent one form of a group of autoimmune diseases (Cudworth & Wolf, 1982). Although Hoover & Capra (1987) found that the number of heterozygotes for TcR-*Cβ Bgl*II polymorphism was significantly increased only in diabetic subjects who were positive for DR3, the present classification of patients based on their HLA-DR types did not reveal any differences. There may still be heterogeneity even within DR3-associated IDDM, of course. DR3-positive patients with other autoimmune diseases are usually older and female; accordingly, these cases may be under-represented in the present series comprising exclusively children and adolescents. No differences were found between patients grouped according to their age and/or sex (data not shown).

The results obtained with the constant region probes do not rule out the importance of TcR region genes as elements predisposing individuals to IDDM. Functionally the most important polymorphism is likely to be found in the variable region of the TcR complex, interacting with both the foreign antigen and the HLA molecule, and constant region polymorphism could reflect mutational changes in the variable gene region due to the linkage between various loci. Recent data do not support the notion of a linkage disequilibrium between *Vβ* and *Cβ* segments, however, and a recombination point evidently lies between these gene complexes (Charmley, Concannon & Gatti, 1988). Therefore there may be a significant TcR-*Vβ* polymorphism which was not detected by the TcR-C*β*-specific cDNA probe or the probe/restriction enzyme combination used in this analysis. *Bgl*II was the only one of several restriction enzymes tested by Robinson & Kindt (1985) which detected polymorphism, demonstrating that the TcR constant region is fairly well conserved. Subsequently, however, a restriction site for *Kpn*I has also been demonstrated (Oksenberg *et al.*, 1988) and mapped to 1711 bp downstream from the polymorphic *Bgl*II site of the *Cβ*2 locus (Pearl *et al.*, 1989); however, due to linkage to the *Bgl*II site it does not add essential information. Future attention should be focused on the variable region itself in the search for a new discriminatory marker for IDDM susceptibility.

ACKNOWLEDGMENTS

We thank Dr T. Mak for his generous gift of the TcR-*Cβ* cDNA probe. We also wish to thank Mrs Eeva-Liisa Heikkinen and Ms Anne Jaakkola for their expert technical assistance and the diabetic and control families for their co-operation. This research was supported by grants from the Foundation for Diabetes Research in Finland and the Alma and K.A. Snellman Foundation, Oulu, Finland.

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