Lavori originali

T-cell receptor polymorphism in primary biliary cirrhosis

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T-cell receptor (TCR) plays a key role in immune regulation and polymorphisms of its genes have been found in association with several autoimmune diseases. No data are available for primary biliary cirrhosis, an autoimmune liver disease the natural history of which is highly variable. We studied a TCR constant beta-2 chain polymorphism in 70 patients affected by primary biliary cirrhosis and in 70 healthy controls. The DNA chains of patients and controls were amplified by means of polymerase chain reaction using primers designed around a Bgl II polymorphic restriction site and digested for restriction fragment length polymorphism analysis. We found a slight increase of the heterozygous genotype in patients compared with controls (49 vs 40%), which became higher if only patients with early disease were considered (60 vs 40%). Heterozygous patients had less severe disease as indicated by a lower Mayo score (5.1 ± 1.2 vs 5.7 ± 1.2 in non-heterozygous). Our data suggest that TCR constant beta-2 polymorphism does not play a key role in modulating the multifactorial etiopathogenesis of primary biliary cirrhosis.
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Key words: **Polymerase chain reaction; Primary biliary cirrhosis; T-cell receptor.**

Introduction

Primary biliary cirrhosis (PBC) is a chronic, autoimmune liver disease with an increased incidence in given families and a highly variable course[1]. An increased incidence of the disease in the close relatives of patients suggests the presence of genetic factors in determining the susceptibility to this pathology[2].

Disease association studies have shown a possible role of the major histocompatibility complex (MHC); the most consistent association has been found with HLA-DR8[3,4].

As in other autoimmune diseases, an abnormal regulation of the immune system is involved and T-cell receptor (TCR), one of the key factors in regulating the immune response, may be involved[5]. Interestingly, an abnormal adaptive immune system has been well documented in PBC[6].

Unlike antibodies that recognize antigens as such, the TCR recognizes antigens as a complex of short peptides bound to a MHC molecule on the surface of the antigen presenting cell. The recognition of the antigen leads to an extensive array of temporally ordered biological responses involving all the T-cells of a given functional phenotype[7]. TCRs are heterodimers composed of either alpha and beta or gamma and delta chains; each chain has a variable region involved in antigen recognition, two chain-specific regions named joining and diversity and a constant region which interacts with the CD3 complex on the T-cell surface[8]. As the diversity of TCR beta chain is generated by a random joining of different genes, polymorphisms in TCR genes are carefully studied in order to determine whether they play a role in the abnormal immune response to antigens, possibly leading to a susceptibility to autoimmune diseases[9].

A polymorphism of the constant region of the beta-2 gene has been investigated in several autoimmune diseases and has been found to be associated with type I diabetes mellitus[10], autoimmune hepatitis[11], immunoglobulin A nephropathy[12] and membranous nephropathy[13]. In these studies the polymorphism was investigated by restriction fragment length polymorphism analysis using Bgl II digestion of the genomic DNA in Southern blot experiments.

At nucleotide sequence analysis of the region, we found the position of the nucleotide change to be 300 bp before the beta-2 chain transcription site[14]. Because this polymorphism is in the promoter region of the TCR beta gene, it could influence gene expression and play a functional role in autoimmune diseases. We studied for the first time this polymorphism in 70 PBC patients and 70 controls, using polymerase chain reaction (PCR) amplification and restriction enzyme digestion. The allele frequencies in patients and controls were determined and the clinical characteristics of the patients as well as their genotypes were discussed.

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Methods

Subjects

The PBC population consisted of 70 patients; 10 mL of whole blood was drawn in order to extract the total DNA to be tested for the presence of the TCR constant beta-2 genotype. We included in the study 35 patients with histological or clinical evidence of liver cirrhosis and 35 with no evidence of advanced disease, who were admitted to our Division from September 1999 to March 2000. The diagnosis of PBC was based on internationally accepted criteria[1]. Eight of the 70 patients were antimitochondrial antibody-negative. As they otherwise met the diagnostic criteria for the disease we considered them as having antimitochondrial antibody-negative PBC, as previously reported[15,16]. All patients had to be negative for hepatitis B surface antigen and for antibodies to hepatitis C virus. The duration of disease was calculated as the time between the date of the earliest suspected evidence of liver disease and the date of blood sampling. The patients with no fibrosis at liver biopsy, i.e. those in Ludwig's stages I and II[17], were considered as having early-stage disease; those with fibrosis or cirrhosis (i.e. stage III or IV) were considered as having advanced disease. The major characteristics of the patients are shown in table I.

Seventy healthy subjects (62 females and 8 males) matched by gender and ethnic origin were chosen as controls among students and laboratory staff. The study protocol followed the ethical guidelines of the 1975 Declaration of Helsinki and subsequent modifications, and all of the patients and healthy controls gave their written consent after being informed about the nature of the study.

T-cell receptor constant beta-2 genotyping

The DNA was extracted by proteinase K digestion, phenol extraction and ethanol precipitation as previously described[18]. PCR was performed with 30 cycles at 93°C for 1 min, 55°C for 1 min and 72°C for 1 min[19], using primers designed around the polymorphic Bgl II site, according to the sequences of the National Center for Biotechnology Information web site program (Gene Bank access number U66061) (forward primer: 5' TAATTTTGAAATAAGGGAAGATGAC 3' - reverse primer: 5' TTTTGTATCCACCCTATGGGTTGGC 3'). The DNA was digested at 37°C for 6 hours as previously described[20] and samples were run on ethidium bromide-stained 2% agarose gel. The PCR amplification product was a 603 bp fragment and restriction with Bgl II gave rise to two fragments of 203 and 400 bp. As the T/C polymorphism may lead to the disappearance of the Bgl II site, the presence of three bands showed heterozygosity of the 603 bp band alone homozygosity for the C nucleotide and of bands of 203 and 400 bp homozygosity for the T nucleotide (Fig. 1).

Statistical analysis

To compare the groups of patients the χ^2 and the Fisher's exact tests were used in the analysis of categorical variables. With regard to the comparison of the genotype frequencies, significance levels were corrected for multiple testing using the Bonferroni inequality method. In this procedure, the p values obtained for each genotype comparison are corrected (p_B) by multiplying by the number of genotypes compared, i.e., by 3. With regard to continuous variables, the Mann-Whitney test was used to compare two groups, and the Kruskal-Wallis non-parametric one-way analysis of variance test to compare more than two groups. Statistical comparisons were made using the Stata Statistical Software (Stata Corporation, College Station, TX, USA). All analyses were two-sided, and p values of < 0.05 were considered statistically significant.

TABLE I. Characteristics of the primary biliary cirrhosis (PBC) patients at the time of blood sampling.

	All patients (n = 70)	PBC		
		Early disease (n = 35)	Advanced disease (n = 35)	p*
No. females	62 (89%)	31 (89%)	31 (89%)	–
Age (years)	59 ± 14	55 ± 13	63 ± 13	0.0157
Total bilirubin (mg/dL; n.v. < 1.0)	0.8 ± 0.4	0.7 ± 0.3	0.9 ± 0.5	–
Albumin (g/dL; n.v. > 3.5)	4.3 ± 0.4	4.4 ± 0.4	4.2 ± 0.5	0.1195
Prothrombin time (INR; n.v. < 1.2)	0.98 ± 0.09	0.97 ± 0.08	0.99 ± 0.10	–
With ascites (n =)	6 (9%)	1 (3%)	5 (14%)	0.1980
With associated diseases** (n =)	20 (29%)	10 (29%)	10 (29%)	–
Mayo score value	5.17 ± 0.84	4.77 ± 0.69	5.57 ± 0.79	0.0004
Disease duration (years)	11 ± 6	10 ± 7	13 ± 6	0.0481

Values are expressed as means ± standard deviation.
* early versus advanced disease (values < 0.2 level are shown); ** autoimmune diseases most frequently associated with PBC (autoimmune thyroid disease, scleroderma, Sjögren's syndrome).

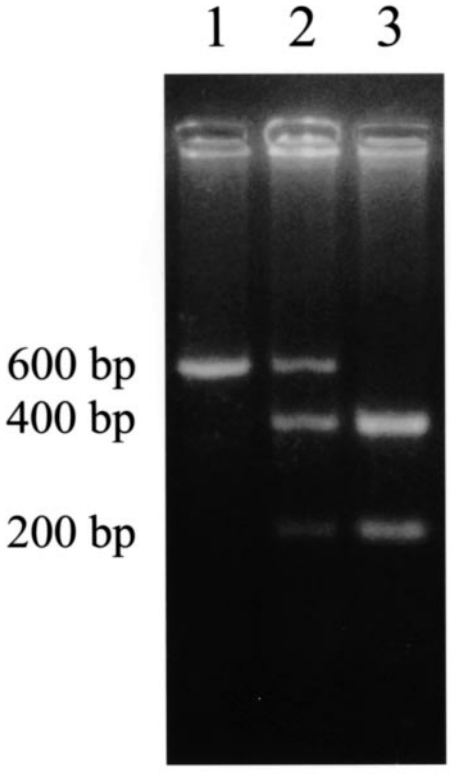

FIGURE 1. *Agarose gel stained with ethidium bromide showing in* lane 1 *the homozygous C pattern (without restriction by Bgl II), in* lane 2 *the heterozygous pattern and in* lane 3 *the homozygous T pattern (complete restriction by Bgl II). The molecular weights of the polymerase chain reaction products are shown.*

Results

Table II shows the TCR constant beta-2 genotypes of the PBC groups. A slight increase in the frequency of the heterozygous status for the T/C residues was found in patients as compared with controls: 49 vs 40%. If only the 35 patients with early stage PBC were considered, the difference became higher (60 vs 40% in controls).

The prevalence of the heterozygous status differed between patients with early and patients with advanced disease (60 vs 37%, p = 0.093); the difference in clinical stage of disease among patients became statistically significant (p = 0.032) when only patients with the T/C and C/C genotypes were compared (Table II). Heterozygous patients (Table III) were significantly younger and had less advanced disease, as highlighted by earlier histologic stage and lower values of the Mayo score, a validated prognostic index developed for PBC[21]. Serum albumin and prothrombin levels were also slightly higher in heterozygous patients.

Discussion

PBC is known to be an autoimmune disease with many factors determining the susceptibility and severity of disease expression[1]. As PBC presents a wide spectrum of severity and of the rate of progression to cirrhosis, with some patients remaining asymptomatic with histologically early disease for several decades after the time of onset[22] and others progressing rapidly to advanced disease, it would be of great interest to determine the genetic marker(s) of disease progression.

We found an association between the T/C genotype and less advanced disease as shown by the differing Mayo score between groups, although the younger age of patients in the T/C group could have exerted a confounding effect.

Several polymorphic loci have recently been studied in order to identify genetic factors which, in association with MHC, may explain the variable susceptibility to PBC and the different progression rates.

Previous evidence showed that TCRs play a fundamental role in the altered immune reaction leading to autoimmune disease development. Several studies demonstrated that TCR constant beta-2 polymorphism may be considered as a factor modulating the progression and severity of autoimmune diseases. In autoimmune hepatitis[11], type I diabetes mellitus[10], immunoglobulin A nephropathy[12] and membranous nephropathy[13] the T/C genotype has been associated with a later onset and with less advanced disease.

In our study we performed TCR constant beta-2 genotyping of PBC patients and found a non-significant allelic distribution between patients and healthy controls;

TABLE II. Distribution of alleles in primary biliary cirrhosis (PBC) patients and controls.

TCR genotype	Controls (n = 70)	All patients (n = 70)	PBC		
			Early disease (n = 35)	Advanced disease (n = 35)	p_B*
T/T (n =)	21 (30%)	21 (30%)	10 (29%)	11 (31%)	–
T/C (n =)	28 (40%)	34 (49%)	21 (60%)	13 (37%)	0.2790
C/C (n =)	21 (30%)	15 (21%)	4 (11%)	11 (31%)	0.2346

TCR = T-cell receptor.
* the significance levels are reported after correction (p_B) for multiple testing.

TABLE III. Biochemical and serological features of primary biliary cirrhosis patients according to the presence of the T-cell receptor C beta-2 polymorphism.

	C/C (n = 15)	T/C (n = 34)	T/T (n = 21)	p*
No. females	13 (87%)	31 (91%)	17 (86%)	–
Age (years)	62 ± 13	54 ± 13	64 ± 12	0.046
With early stage (n =)	4 (29%)	21 (60%)	10 (11%)	0.074
With advanced stage (n =)	11 (31%)	13 (37%)	11 (31%)	–
Total bilirubin (mg/dL; n.v. < 1.0)	0.9 ± 0.6	0.8 ± 0.3	0.6 ± 0.2	–
Albumin (g/dL; n.v. > 3.5)	4.3 ± 0.4	4.4 ± 0.4	4.1 ± 0.5	–
Prothrombin time (INR; n.v. < 1.2)	0.96 ± 0.12	1.01 ± 0.09	0.96 ± 0.05	–
Ascites (n =)	2 (13%)	2 (6%)	2 (10%)	–
Associated diseases** (n =)	5 (36%)	9 (26%)	6 (29%)	–
Mayo score value	5.3 ± 0.8	4.99 ± 0.84	5.4 ± 0.8	0.158
Disease duration (years)	11 ± 7	11 ± 7	12 ± 6	–

Values are expressed as means ± standard deviation.
* the significance levels (p) of the comparison between heterozygous and non-heterozygous primary biliary cirrhosis patients are reported (values < 0.2 level are shown); ** autoimmune diseases most frequently associated with primary biliary cirrhosis (autoimmune thyroid disease, scleroderma, Sjögren's syndrome).

however some differences between early and advanced disease were found. This finding could be explained by the role played by other factors (i.e. MHC) in determining the disease susceptibility; thus the PBC population may be genetically different from normal controls. The allelic association with different stages of the disease may be due to a gene dosage effect caused by the different nucleotide in the promoter region which, in turn, could explain the lower prevalence of the homozygous C genotype and the higher prevalence of the heterozygous T/C genotype in early stage PBC found in our study. A semiquantitative PCR analysis to determine the exact amount of the TCR beta could support this hypothesis.

Riassunto

Il recettore dei linfociti T (TCR) svolge un ruolo fondamentale nella regolazione del sistema immunitario e polimorfismi dei suoi geni sono stati descritti in associazione ad alcune malattie autoimmuni. Non sono disponibili dati relativi a polimorfismi del TCR in pazienti con cirrosi biliare primitiva, una malattia autoimmune del fegato caratterizzata da una grande variabilità della sua storia naturale. Abbiamo studiato un polimorfismo della catena beta-2 costante del TCR in 70 pazienti affetti da cirrosi biliare primitiva e 70 controlli sani. DNA di pazienti e di controlli è stato amplificato mediante reazione polimerasica a catena usando *primers* disegnati intorno al sito polimorfico di restrizione Bgl II e digerito per l'analisi del polimorfismo della lunghezza dei frammenti di restrizione. È emerso un modesto aumento del genotipo eterozigote nei pazienti rispetto ai controlli (49 vs 40%), che diventava più marcato se si consideravano solo i pazienti con malattia in stadio iniziale (60 vs 40%). I pazienti eterozigoti avevano una malattia meno severa come indicato da un più basso punteggio Mayo (5.1 ± 1.2 vs 5.7 ± 1.2 nei non eterozigoti). I nostri dati suggeriscono che il polimorfismo della catena beta-2 costante del TCR sembra non svolgere un ruolo importante nel modulare l'eziopatogenesi multifattoriale della cirrosi biliare primitiva.

Parole chiave: Cirrosi biliare primitiva; Reazione polimerasica a catena; Recettore dei linfociti T.

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References

1. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1996; 335: 1570-9.
2. Tanaka A, Borchers AT, Ishibashi H, Ansari AA, Keen CL, Gershwin ME. Genetic and familial considerations of primary biliary cirrhosis. Am J Gastroenterol 2001; 96: 8-15.
3. Underhill J, Donaldson P, Bray G, Doherty D, Portmann B, Williams R. Susceptibility to primary biliary cirrhosis is associated with the HLA-DR8-DQ9B1*0402 haplotype. Hepatology 1992; 16: 1404-8.
4. Manns MP, Kruger M. Immunogenetics of chronic liver diseases. Gastroenterology 1994; 106: 1676-97.
5. Mackelprang R, Carlson CS, Subrahmanyan L, Livingston RJ, Eberle MA, Nickerson DA. Sequence variation in the human T-cell receptor loci. Immunol Rev 2002; 190: 26-39.
6. Kita H, Mackay IR, Van de Water J, Gershwin ME. The lymphoid liver: considerations on pathways to autoimmune injury. Gastroenterology 2001; 120: 1485-1501.
7. Lanzavecchia A. Identifying strategies for immune intervention. Science 1993; 260: 937-44.

8. Ashwell JD, Klausner DR. Genetic and mutational analysis of the T- cell antigen receptor. Ann Rev Immunol 1990; 8: 139-67.

9. Weissman SM. Genetic bases for common polygenic diseases. Proc Natl Acad Sci USA 1995; 92: 8543-4.

10. Millward BA, Welsh KI, Leslie RD, Pyke DA, Demaine AG. T cell receptor beta chain polymorphisms are associated with insulin-dependent diabetes. Clin Exp Immunol 1987; 70: 152-7.

11. Manabe K, Hibberd ML, Donaldson PT, et al. T-cell receptor constant beta germline gene polymorphisms and susceptibility to autoimmune hepatitis. Gastroenterology 1994; 106: 1321-5.

12. Tatsuma N, Tsugu H, Murakami M. HLA-DQ region and TCR gene polymorphism associated with primary IgA nephropathy in Japanese children. Nippon Jinzo Gakkai Shi 1997; 39: 734-9.

13. Demaine AG, Vaughan RW, Taube DH, Welsh KI. Association of membranous nephropathy with T cell receptor constant beta chain and immunoglobulin heavy chain switch region polymorphisms. Immunogenetics 1988; 27: 19-23.

14. Tripputi P, Graziani D, Alfano RM, Cassani B, Coggi G. A common T/C polymorphism in the promoter region of the beta T-cell receptor gene. Mol Cell Probes 2000; 14: 195-7.

15. Invernizzi P, Crosignani A, Battezzati PM, et al. Comparison of the clinical features and clinical course of antimitochondrial antibody-positive and -negative primary biliary cirrhosis. Hepatology 1997; 25: 1090-5.

16. Invernizzi P, Battezzati PM, Crosignani A, et al. Antibody to carbonic anhydrase II is present in primary biliary cirrhosis (PBC) irrespective of antimitochondrial antibody status. Clin Exp Immunol 1998; 114: 448-54.

17. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 1978; 379: 103-12.

18. Tripputi P, Guerin S, Moore DD. Two mechanisms for the extinction of gene expression in hybrid cells. Science 1988; 241: 1205-7.

19. Tripputi P, Bacciocchi G, Accolla RS, et al. Detection of human chromosomes in somatic cell hybrids by PCR analysis. Cytogenet Cell Genet 1993; 62: 1-4.

20. Tripputi P, Emanuel BS, Croce CM, Green LG, Stein GS, Stein JL. Human histone genes map to multiple chromosomes. Proc Natl Acad Sci USA 1986; 83: 3185-9.

21. Dickson ER, Grambsch P, Fleming TR, Fisher LD, Langworthy A. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989; 10: 1-7.

22. Metcalf JV, Mitchison HC, Palmer JM, Jones DE, Bassendine MF, James OF. Natural history of early primary biliary cirrhosis. Lancet 1996; 348: 1399-402.

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