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THE T-CELL RECEPTOR ALPHA, BETA AND GAMMA POLYMORPHISM IN JAPANESE

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Summary Polymorphism in the genes encoding the alpha (α), beta (β) and gamma (γ) chains of the human T-cell receptors was analyzed both in population and family studies. Against twelve unrelated Japanese, several out of the 15 restriction endonucleases tested, revealed restriction fragment length polymorphism. The segregation of the polymorphic fragments were confirmed among 15 members of three families. In most of the cases paternal and/or maternal haplotypes could be assigned. By testing the polymorphic enzymes among the random healthy Japanese, the frequency of each polymorphic fragment was then determined. Although the polymorphism found in this study was similar to that reported in Caucasians, some differences were observed. Such differences are discussed. The restriction fragment length polymorphism in both population and family studies, derived from α, β and γ chains of the T-cell receptor found in this report, might be useful markers for genetic analysis of the T-cell function in relation to immunological disorders.

Key Words restriction fragment length polymorphism (RFLP), T-cell receptors α, β and γ chains

INTRODUCTION

T-cell antigen receptor (TCR), a cell surface protein heterodimer consisting of an acidic α, a basic β, a third γ (Haskins *et al.*, 1983, Meuer *et al.*, 1983; Saito *et al.*, 1984) and a fourth δ chain, have recently been cloned, allowing the use of restriction fragment length polymorphism (RFLP) for a better understanding of the structure and organization of the TCR genes (Kronenberg *et al.*, 1986).

The analysis of genes polymorphism of the TCR involved in immune processes

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has provided new markers for the study of genetic variability in the immune response of several autoimmune diseases (Hoover *et al.*, 1986a, b; Demaine *et al.*, 1986, 1988; Millward *et al.*, 1987; Vladutiu and Rose, 1975; Wofsy *et al.*, 1985; Bottazzo *et al.*, 1985; Goldstein *et al.*, 1987).

This study describes the results obtained by TCR α, β and γ restriction fragment length polymorphism (RFLP) analysis in Japanese, that is which enzyme shows polymorphism and also the size and frequency of such polymorphic fragments within groups of healthy individuals. An attempt was also made to confirm the segregation of these polymorphism by using three families.

Although T cell receptors has been widely studied among Caucasians (Robinson and Kindt, 1985a, b; So *et al.*, 1987), few investigation has been performed in Japanese. Therefore, we believe that the polymorphism and segregation described here might be useful for further genetic studies especially in relation to the susceptibility of immunological disorders.

MATERIALS AND METHODS

1. Population and family studies. At the beginning, twelve unrelated Japanese were used to find the polymorphic enzyme in relation to each gene. The segregation of this polymorphism was confirmed by using three families, which were consisted with parents and several children. Up to 46 unrelated individuals were used to determine the frequency of the polymorphic fragments, confirmed in the family studies by the different polymorphic enzymes.

2. cDNA probes. The three T-cell receptor cDNA probes used in this study were kindly provided by Dr. Tak Mak.

The T-cell receptor α chain probe was obtained from the human cDNA clone pY14 digested with *Eco*RI. The pY14 clone contains an insert of 1,101 pb that corresponds to the 5′-untranslated region, leader sequence, V, D, J and C regions and 3′-untranslated sequences (Yanagi *et al.*, 1985).

The T-cell receptor β chain probe, a 770 bp, was cloned into *Pst*I site of pBR-322. This probe contains J and C regions of TCR β gene (Yanagi *et al.*, 1984).

The T-cell receptor γ chain probe, a 1.6 kb insert containing V, J and C regions were cloned into *Eco*RI site (Yoshikai *et al.*, 1987). *Hin*dIII was used to separate variable (V) and joining (J) fragments from the constant (C) region.

3. RFLP analysis. RFLP analysis was performed as previously reported (Aparicio *et al.*, 1988). Fifteen enzymes (*Bgl*I, *Hae*II, *Eco*RI, *Hin*dIII, *Msp*I, *Hae*III, *Hin*fI, *Hin*cII, *Sty*I, *Pst*I, *Xba*I, *Pvu*II, *Bgl*II (at 37°C), *Bam*HI (at 30°C) and *Taq*I (at 65°C) were used to find the polymorphic enzymes.

4. Statistical analysis. Allelism of the polymorphic fragments were confirmed by chi-square analysis whether the distribution of the given fragment combination were in Hardy-Weinberg's equilibrium.

RESULTS AND DISCUSSION

Since several disorders has been found to have an autoimmune basis, T-cell receptors of the lymphocytes have been widely investigated. For example, the T-cell receptor β was observed to be associated with insulin dependent diabetes mellitus in Caucasians (Hoover *et al.*, 1986a, b; Demaine *et al.*, 1986, 1988; Millward *et al.*, 1987) differently than in Japanese (Aparicio *et al.*, submitted for publication). This report demonstrates RFLP analysis of the possible genes encoding for α, β and γ chain of the human T-cell receptors in Japanese. A summary of the polymorphic enzymes found here is shown in Table 1.

Polymorphism in the T-cell receptor α gene

By means of Southern blot analyses, twelve DNA samples were digested with 15 restriction endonucleases by using a probe that correspond to the human T-cell receptor α chain (Yanagi *et al.*, 1985). Three out of the 15 enzymes presented

Table 1. Polymorphic restriction enzymes and their respective fragments for the T-cell receptor α, β, and γ in Japanese.

Probe/enzyme	Polymorphic fragments		Invariant fragments (kb)
	Single (kb)	Diallelic (kb)	
TCR α			
*Taq*I	(1.4)	7.4/2.2	3.5, 2.6, 1.5, 1.2, 0.9
*Pvu*II[b]	—	5.2/4.3	7.8, 4.9, (1.2), (1.1)
*Eco*RI[b]	13.2	—	16.5, 8.4, 2.7, 2.1
TCR β			
*Bgl*II	—	9.0/8.4	2.3, 1.3
*Taq*I	7.0[a]	—	5.0, 3.4, (2.3), 1.7
*Bam*HI	11.5[a]	—	21.0
TCR γ			
*Taq*I	—	2.8/2.1(C)	6.2(C), 3.9(C), 2.5(C)
*Xba*I	3.9(C)	7.0/6.7(VJ)	10.0(C), 3.5(C), 3.2(C), 2.3(C)
*Msp*I	—	7.2/5.2(C), 1.2/1.0 (VJ)	4.7(C), 4.3(C), 3.9(C)
*Pst*I	3.7(C)	—	4.1(J), 2.3(C), 1.8(C), 1.5(C)
*Pvu*II[b]	10.0(C)	—	11.2(C), 8.0(C), 3.2(VJ)
*Bgl*I[b]		21.0/12.0(VJ), 19.0/4.4(C)	—

( ) faint bands. [a] Polymorphic fragments which did not segregate to the offsprings. [b] Enzymes which were not tested among the population studies. (C), fragments derived from the constant region. (V,J), fragments derived from the variable and joining regions.

polymorphic fragments. They are, *Taq*I (7.4, 3.5, 2.6, 2.2, 1.5, 1.4, 1.2, 0.9 kb), *Pvu*II (7.8, 5.2, 4.9, 4.3, 3.3, 1.2, 1.1 kb) and *Eco*RI (16.5, 13.2, 8.4, 2.7, 2.1 kb). Among these fragments, the 7.4 and 2.2 kb of *Taq*I showed mutually allelic distribution. This allelism was confirmed by family and population studies, that is the number of individuals carrying these specific fragment combinations were similar to those expected by Hardy-Weinberg's equilibrium. 5.2 and 4.3 kb of *Pvu*II fragments were also suggested to be allelic by family segregation.

Table 2 shows the frequency of *Taq*I among a group of healthy individuals.

The polymorphic fragments were compared to those reported in Caucasians,

Table 2. Frequency of the T-cell receptor α, β and γ among healthy Japanese.

	Enzyme	Fragment size	Frequency	
			Number	%
TCRα				
	*Taq*I	7.4 / 7.4	3 / 24	12.5
		7.4 / 2.2	12 / 24	50.0
		2.2 / 2.2	9 / 24	37.5
		Total	24	
		1.4	9 / 24	37.5
TCRβ				
	*Bgl*II	9.0 / 9.0	1 / 23	4.3
		9.0 / 8.4	9 / 23	39.1
		8.4 / 8.4	13 / 23	56.5
		Total	23	
TCRγ				
	*Taq*I	2.8 / 2.8	3 / 24	12.5
		2.8 / 2.1	10 / 24	41.6
		2.1 / 2.1	11 / 24	45.8
		Total	24	
	*Pst*I	3.7	3 / 16	18.7
	*Xba*I	7.0 / 7.0	0 / 53	0.0
		7.0 / 6.7	8 / 53	15.1
		6.7 / 6.7	45 / 53	84.9
		Total	53	
		3.9	23 / 53	43.4
	*Msp*I	7.2 / 7.2	5 / 38	13.2
		7.2 / 5.2	25 / 38	65.8
		5.2 / 5.2	8 / 38	21.1
		Total	38	
		1.2 / 1.2	8 / 38	21.1
		1.2 / 1.0	22 / 38	57.9
		1.0 / 1.0	8 / 38	21.1
		Total	38	

as an example, although there is a small variation on the molecular weight of the *Taq*I polymorphic 7.4, 2.2 and 1.4 kb fragments, they seem to be similar to those 8.0, 2.1, 1.0 (Robinson and Kindt, 1987) or 7.0, 2.0 and 1.4 kb (So *et al.*, 1987) fragments found in Caucasians. Moreover, it was demonstrated by hybridizing with a Cα probe, that the allelic polymorphism of 8.0 and 2.1 kb bands (7.4 and 2.2 kb in our study), seem to be due to changes in restriction sites around the exon 1 of C gene, whereas the 1.0 kb (1.4 kb in our study) due to the polymorphism of the V gene (Robinson and Kindt, 1987; So *et al.*, 1987). On the contrary, polymorphic fragments of *Msp*I-4.5 kb and *Bgl*II-3.0 kb found in Caucasians were observed as invariant chains of 4.3 and 2.8 kb respectively in Japanese.

Polymorphism in the T-cell receptor β genes

By using a probe which correspond to the C region of the β chain (containing J and C regions) of the human T-cell receptor (C) (Yoshikai *et al.*, 1984), where only 3 enzymes showed polymorphism. *Bgl*II (9.0, 8.4, 2.3, 1.3 kb), *Taq*I (7.0, 5.0, 3.4, 2.3, 1.7 kb) and *Bam*HI (21.0, 11.5 kb). The *Bgl*II-9.0/8.4 kb fragments were allelic, and seems to be similar to those reported in Caucasians (Robinson and Kindt, 1985a, b). Polymorphic fragments of *Bam*HI (11.5 kb) and *Taq*I (7.0 kb) showed faint bands and family segregation was not confirmed (Fig. 1, A and B, respectively). These unexpected segregation of the TCR β genes were also

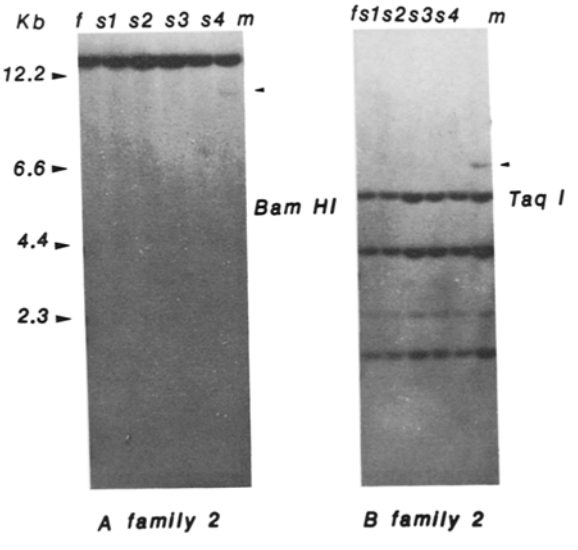

Fig. 1. Segregation of the TCR β RFLP in family 2. The donors of DNA in the gel lanes are, father (f), mother (m) and children 1–4 (s1–s2) in this family. A) DNA samples were digested with *Bam*HI, B) with *Taq*I and then hybridized with TCR β J and C regions probe (Yanagi *et al.*, 1984).

reported in Caucasians (Robinson *et al.*, 1985a), due to rearrangement or some other alteration in the germ line configuration. They found a *Bam*HI-15.0 kb fragment in one of the children, although both parents lacked it. The relationship between *Bam*HI 11.5 kb polymorphic fragment found in this study, and the 15.0 kb fragment in Robinson's were unclear.

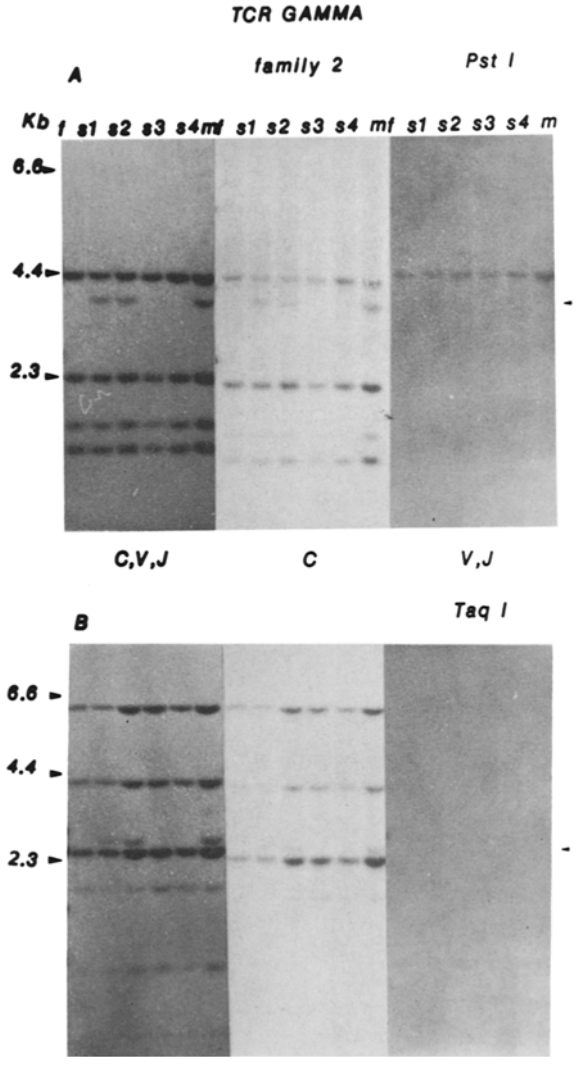

Fig. 2. Segregation of the TCRγ RFLP in family 2. The donors of DNA in the gel lanes are, father (f), mother (m) and children 1–4 (s1–s2) in this family. A) DNA samples were digested with *Pst*I, B) with *Taq*I. All the blot was hybridized with TCRγ V,J and C regions probe (Yoshikai *et al.*, 1987). The samples were also re-hybridized with the constant region (C) and variable and joining regions (V, J) separately.

Table 2 shows the frequency of *Bgl*II among a group of healthy individuals.

Polymorphism in the T-cell receptor γ gene

Restriction fragment length polymorphism associated with the T-cell receptor γ which correspond to the V, J and C region (Yoshikai *et al.*, 1987) was also studied among the unrelated Japanese and three families. Moreover, in order to find more precisely the TCR γ probes were divided into 2 fragments, the V and J regions of the TCR γ and that of C region. These specific probes were used separately to determine the origins of the fragments.

TCR γ polymorphic fragments were observed in 6 enzymes; *Xba*I (10.0, 7.0, 6.7, 3.9, 3.5, 3.2, 2.3 kb), *Taq*I (6.2, 3.9, 2.8, 2.5, 2.1 kb), *Pst*I (4.1, 3.7, 2.3, 1.8, 1.5 kb), *Msp*I (7.2, 5.2, 4.7, 4.3, 3.9, 1.2, 1.0 kb), *Pvu*II (11.2, 10.0, 8.0, 3.2 kb), *Bgl*I (21.0, 19.0, 12.0, 4.4 kb). Among these fragments, allelism was also present, the *Taq*I-2.8 kb / 2.1 kb, *Xba*I-7.0 kb / 6.7 kb. In addition, two di-allelic polymorphism, 21.0 kb / 12.0 kb, 19.0 kb / 4.4 kb and 7.2 kb/ 5.2 kb, 1.2 kb / 1.0 kb, were demonstrated when *Bgl*I and *Msp*I were applied respectively. The polymorphic *Pvu*II-10.0 kb fragment in Japanese differs completely from allelic polymorphism of *Pvu*II-20.0, 17.0 and 15.0 kb fragments found in Caucasians (Dunckley *et al.*, 1988). *Pst*I and *Taq*I polymorphism, so far as we know, of TCR γ has not reported yet in Caucasian which we could confirm in Japanese. As an example, segregation of *Pst*I and *Taq*I fragments in family 2 were shown in Fig. 2, A and B, where the polymorphic *Pst*I-3.7 kb and *Taq*I-2.9 kb fragments were inherited by two children from the mother. These results indicated some differences might be present in TCR genes among the ethnic groups.

The frequencies of *Msp*I, *Taq*I, *Pst*I, *Bgl*I, and *Xba*I polymorphic fragments are shown in Table 2.

By means of RFLP, the genes encoding the α, β and γ chains of the human T-cell receptors and their segregation among families have been analyzed. The purpose of this study was then to find the possible polymorphism in Japanese and to analyze their frequency. Therefore, we believe that this polymorphism could provide useful markers, which together with the restriction fragment length polymorphism of the HLA region might contribute to a better understanding of the gene complex which is responsible for the development of the various immunological disorders.

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