DNA typing from formalin-fixed, paraffin-embedded tissues

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INTRODUCTION

Serologically defined individual variation has been shown to be considerable in HLA class I and class II. Recently, HLA alleles were defined using polymerase chain reaction(PCR-DNA typing). As previously reported(Ota 1991), the PCR-restriction fragment length polymorphism(PCR-RFLP) method is the most useful to define the HLA-DQA1, DQB1, DRB1 and DPB1 alleles. The advantage of this method is the use of some informative restriction enzymes, which have either a single cleavage site or alternatively no cleavage site in the amplified DNA region.

In this study, we attempted to extract DNA from old formalin-fixed, paraffin-embedded samples of tissues, such as brain, heart, lung, liver and kidney. The extracted DNA was amplified and typed using the PCR-RFLP method.

MATERIALS AND METHODS

For comparison with DNA extraction from (1)long-term, formalin-fixed and embedded samples, and (2)short-term, formalin-fixed and long-term embedded samples, brain ,heart, lung, liver and kidney tissues were fixed in phosphate buffered formaldehyde (3-5%) or unbuffered formaldehyde(3-5%) at room temperature for two months or two years. 5-μ sections were cut from blocks and deparaffinized by immersion for 30 minutes at room temperature in 1ml of xylene, spun 5 minutes in a microfuge, and decanted (Shibata 1988). This procedure was repeated. The sections were rinsed once with 100% ethanol by inverting the tube two or three times. The samples were then centrifuged and the liquid decanted. The procedure was repeated with 100% ethanol. The last rinse was decanted and the remaining ethanol evaporated under vacuum. The sections were resuspended in 300μl of digestive buffer(10mM Tris-HCl,pH8.0, 10mM EDTA, 0.1M NaCl) containing 1%SDS and proteinase K(250μg/ml). The mixture was incubated for over 5 hours at 50°C. After the solution was extracted with water-saturated phenol, TE buffer was added. The solution was concentrated on a Centricon 30 microconcentrator(Amicon)(Pääbo 1988). After addition of distilled water and a second concentration, the solution was used for the DNA template.

For PCR amplification, the reaction mixture was subjected to 30 cycles of 1 minute at 92°C, 1 minute at 62°C, and 2 minutes at 72° by automated PCR thermal cyclers. The DQA1 gene was amplified

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using the PCR primers GH26 and GH27 at 1 µM. After amplification, aliquots of the reaction mixtures were digested with the restriction endonucleases, ApaLI, HphI, BsaJI, FokI and MboII, for 5 hours after adding the appropriate incubation buffer. Samples of the restriction-enzyme-cleaved amplified DNAs were subjected to electrophoresis in 12% polyacrylamide gel in a minigel apparatus. Restriction fragments were detected by staining with ethidium bromide.

RESULTS AND DISCUSSION

Genetic characterization of individuals for identity testing is being performed increasingly at the DNA level. A new, technically more feasible strategy for individual identification at the DNA level is the use of PCR. The advantage of the PCR technique is that it enables the analysis of minute amounts of DNA from various sources, including impure or degraded samples (Higuchi 1988). DNA analysis from the formalin-fixed, paraffin-embedded tissues was performed by the PCR procedure with Tag DNA polymerase. This method has been applied successfully to DNA extracted from 2-year-old paraffin blocks as well as from 2-year-old formalin-fixed tissue stored at room temperature (Table 1). Although it is reported that unbuffered formalin markedly degrades DNA, our procedure shows that amplification of short specific DNA sequences is still possible.

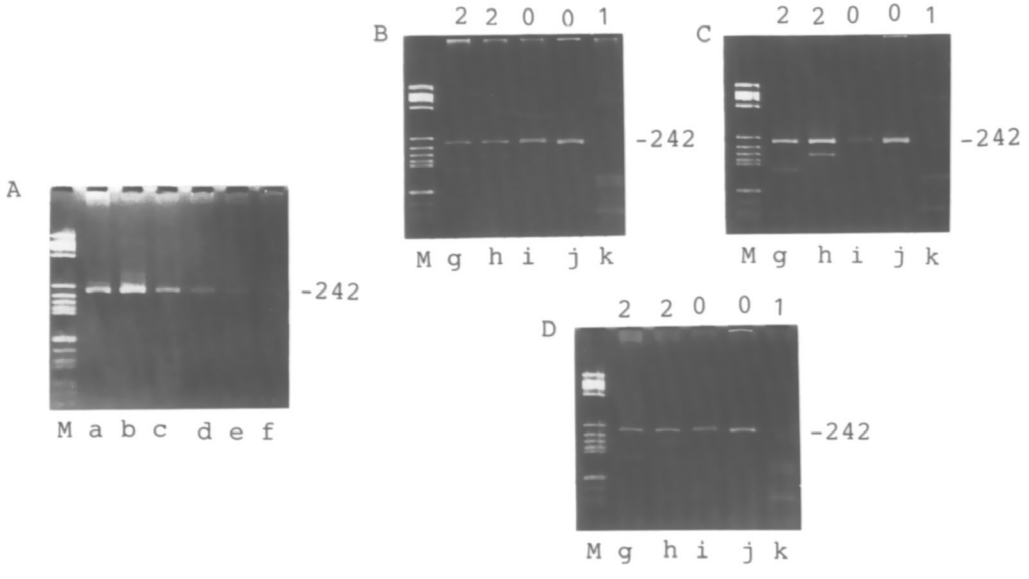

Fig. 1 PCR amplification and restriction analysis of HLA-DQA1 fragments in various organs(buffered formalin)

A:amplified DNA	B:brain	g:ApaLI	0:not cleaved
a,d:brain	C:heart	h:HphI	1:cleaved
b,e:heart	D:liver	i:BsaJI	
c,f:liver		j:FokI	
1-week-old(a,b,c)		k:MboII	
2-month-old(d,e,f)			

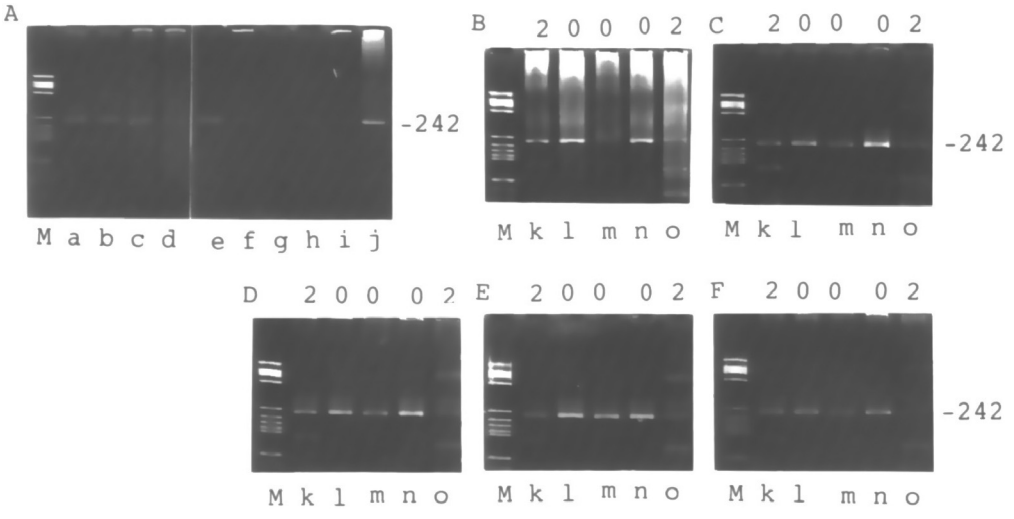

Fig. 2 PCR amplification and restriction analysis of HLA-DQA1 fragments in various organs(unbuffered formalin)
A:amplified DNA B:blood k:ApaLI 0:not cleaved
a,e:brain, b,f:heart C:brain l:HphI 1:cleaved
c,g:liver, d,h:kidney D:heart m:BsaJI
i:lung, j:blood E:liver,F:kidney n:FokI,o:MboII
2-year-old paraffin-embedded(a,b,c,d)
2-year-old formalin-fixed(e,f,g,h)

Table 1. HLA-DQA1 genotyping from tissues using PCR-RFLP

	Genotype	Restriction endonucleases				
		ApaLI	HphI	BsaJI	FokI	MboII
case I(2-month-old)	0103/0301	2	2	0	0	1
case II(2-year-old)	0102/0301	2	0	0	0	2

0: not cleaved, 1:cleaved, 2:both for heterozygote. DQA1 0101 and 0102 can be discriminated by RFLP bands (23 bp and/or 40 bp) digested with MnlI enzyme. case I and case II: blood, brain, heart, liver, kidney, lung.

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