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Analyses of Gene Structures and Antigen Determinants of Human Class II Major Histocompatibility Antigens

II. Subtype Frequencies in Type I Diabetic Patients with DR2 Haplotype*

JUN KAWAI

Shionogi Research Laboratories, Shionogi & Co., Ltd.,
Fukushima-ku, Osaka 553, Japan

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Abstract The serologically defined HLA-DR2 specificity is divided into several Dw subtypes determined by the mixed leukocyte reaction, Dw2, Dw12, Dw21 and Dw blank. HLA-DR2 allele is negatively associated with insulin-dependent diabetes (IDDM) in Japanese, suggesting that resistance related factors are involved in DR2 haplotype. It is still unknown, however, which Dw specificities in DR2 correlate with that resistance. I investigated the genomic clones and restriction fragment length polymorphism (RFLP) from DR2 specificities to show an ability of Dw typing in DR2 by genomic Southern blotting with the DQβ cDNA probe. Furthermore, I compared Dw subtype frequencies in IDDM patients carrying DR2 haplotype to that of healthy individuals in Japanese, but failed to observe statistical significance.

INTRODUCTION

The major histocompatibility complex (MHC) codes for at least three classes of products involved in the immune response (class I, class II, and class III) (Klein et al., 1983). Class II products are membrane glycoproteins composed of two chains, α and β. Three class II products, DR, DQ, and DP antigens, which respectively are encoded by their genes, HLA-DR, HLA-DQ, and HLA-DP genes, are expressed on antigen presenting cells, such as, B-cells, macrophages, and dendritic cells.

IDDM is resulted from the selective destruction of insulin-producing islet cells of the pancreas. It has been argued that disease development involves an autoimmune response to an islet antigen(s) (Eisenbarth, 1986). The IDDM is clearly polygenic in inheritance (Thomson et al., 1984) and it has been estimated that the HLA-D region contributes to much of the heritability (Rotter and Landlaw, 1984). About 95% of IDDM patients possess either HLA-DR3 or-DR4, compared to 45–54% of the normal population in Caucasian (Svejgaard et al., 1983; Thomson et al., 1984). At least one IDDM susceptibility gene was mapped in the MHC, especially in the HLA-D region, by population and family studies (Winter et al., 1987; Bertrams et al., 1984; Winearls et al., 1984; Rubinstein et al., 1981; Bertrams et al., 1984). The serological (Kim et al., 1983; Schreuder et al., 1985) and RFLP (Owerback et al., 1983; Arnheim et al., 1985; Bohme et al., 1986; Nepom et al.,

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1986) studies of MHC class II antigens have indicated that the HLA-DQ genes, which are in linkage disequilibrium with HLA-DR, are more strongly associated with IDDM than DR genes. In Japanese, IDDM is associated with DR4 and DR9, and negatively associated with DR2 (Svejgaard et al., 1984; Bertrams and Baur 1984).

I have studied the gene structure and antigenicity in AKIBA (DR2,DQw1,Dw12) and PGF(DR2,DQw1,Dw2) by genomic clone analysis and gene transfection techniques in accompanying paper (Kawai 1990), and demonstrated the possibility of determination of Dw subtypes in DR2. In the present report, I analyzed class II α and β chain RFLPs obtained from the genomic DNAs of HLA-DR2 homozygous typing cells (HTCs), and determined Dw subtypes of DR2 healthy individuals, as well as HLA-DR2 IDDM patients.

MATERIALS AND METHODS

Table 1 shows the DR2-associated homozygous typing cell lines used in the present study. Fourteen healthy individuals and fifteen IDDM patients carrying the DR2 haplotype were studied. Technique for Southern hybridization have been described in the accompanying paper (Kawai, 1990). Probes used for the detection of class II MHC genes were three almost full length cDNAs: pDCα07 for the DQα gene, pDCβ101 for DQβ gene, and pDRβ102 for the DRβ gene (see Kawai, 1991).

RESULTS AND DISCUSSION

Detection of polymorphic fragments specific for HLA-Dw specificities

Serological DR haplotypes are divided into several Dw subtypes determined by the mixed lymphocyte reaction (Back, 1985), DR2 into Dw2, Dw12, Dw21 and Dw blank. Genomic cosmid clone analysis of DR2 specificities showed RFLPs between Dw2(PGF) and Dw12(AKIBA) (Kawai, 1990). I analyzed the possibility of Dw typing by Southern blotting using HLA class II gene cDNAs as probes in representative DR2 homozygous typing cell lines including Dw2, Dw12 and Dw21. In an attempt to establish standard Southern blot patterns for different Dw specificities with HLA class II antigen probes, genomic DNAs carrying DR2 specificity were digested with various restriction endonucleases (EcoRI, PstI, BamHI, TaqI and MspI) and probed with the HLA-DRβ, DQα, and DQβ cDNA probes. Fig.1-3 showed the polymorphic restriction fragments of some DNA samples from three Dw subtypes of DR2 (Dw2, Dw12 and Dw21) hybridizing

Table 1. DR/Dw haplotypes of HTCs.

	HLA	
HTC	DR	Dw
PGF	DR2	Dw 2
Ms-7	DR2	Dw 2
Ci	DR2	Dw 2
AKIBA	DR2	Dw12
TOK	DR2	Dw12
FJO	DR2	Dw21

the DQα, DQβ and DRβ cDNA probes, respectively, after restriction endonucleases digestion. Multiple hybridizing fragments were encountered in DNA from all homozygous typing cell lines with the DRβ, and DQα and DQβ cDNA probes. The Southern blot patterns were illustrated in Fig.4 shematically. Each individual homozygous typing cell lines corresponded to a characteristic pattern of hybridizing fragments regardless of whether the DRβ, the DQβ or the DQα cDNA probe was used. Hybridization with the DRβ and DQα cDNA probe generally gave identical patterns with DNA from different Dw specificities carrying an identical DR type (DR2). In contrast, the DQβ cDNA could easily distinguish different Dw specificities. Hybridization of EcoRI-digested DNAs with the DRβ probe gave six bands, 20, 9.0, 6.0, 5.0, 4.0 and 3.4kb

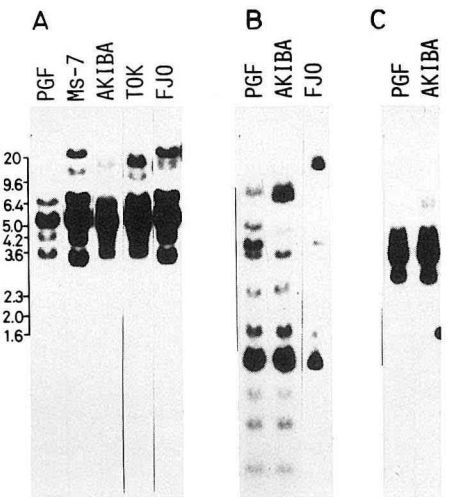

Fig. 1. Autoradiograms of blot hybridization analysis of HLA homozygous DR2-positive B cell lines with the DRβ cDNA, pDRβ101, as probe after digestion of EcoRI(A), PstI(B) and BamHI(C).

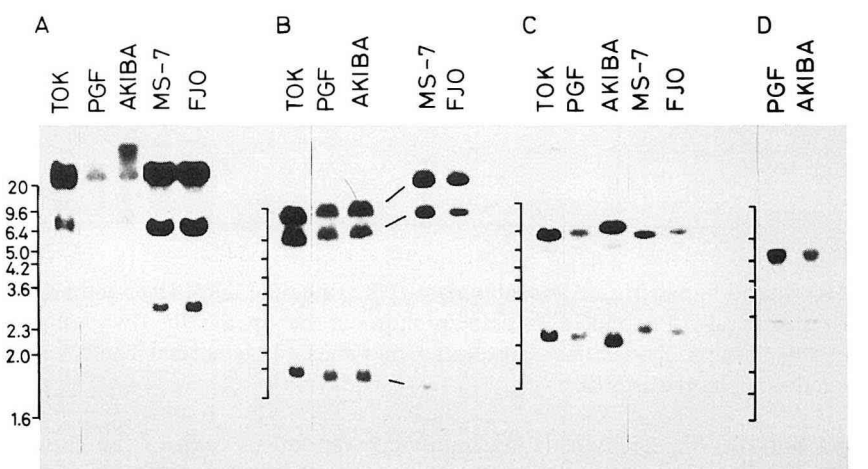

Fig. 2. Autoradiograms of blot hybridization analysis of HLA homozygous DR2-positive B cell lines with the DQα cDNA, pDCα07, as probe after digestion of EcoRI(A), PstI(B), TaqI(C) and MspI(D)

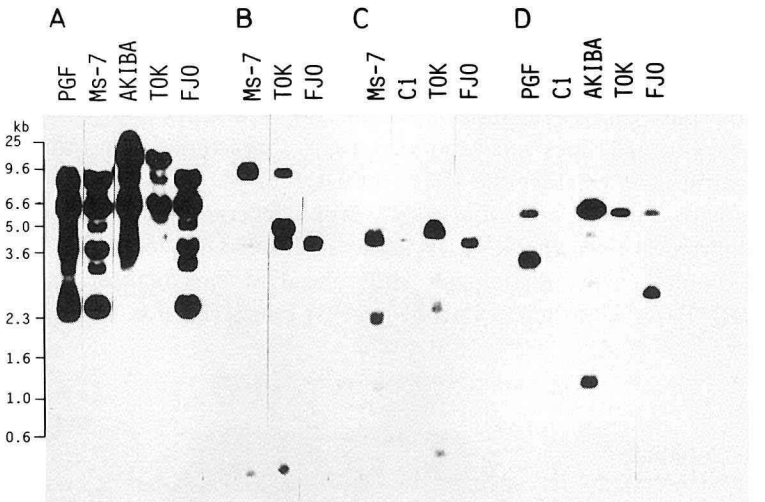

Fig. 3. Autoradiograms of blot hybridization analysis of HLA homozygous DR2–positive B cell lines with the DQβ cDNA, pDCβ101, as probe after digestion of EcoRI(A), PstI(B) MspI(C) and TaqI(D).

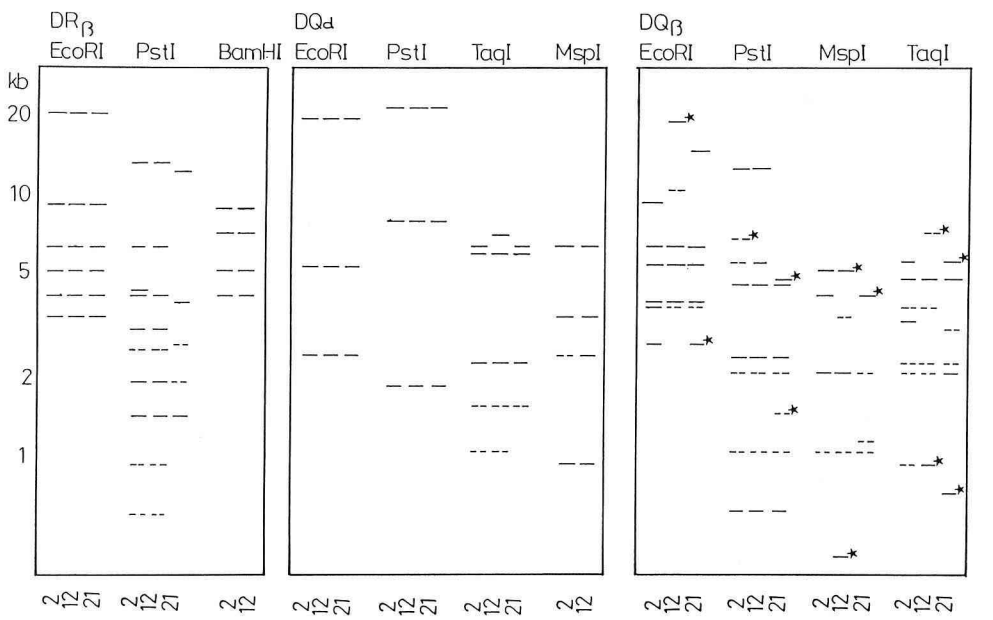

Fig. 4. Hybridization patterns of Dw subtypes of DR2 using the DRβ, DQα and DQβ probes are represented shematically. Endonucleases used are shown at the top, and the Dw specificities of DR2 are shown at the bottom. Dotted lines represents faint bands. Polymorphic bands selected for Dw typing are indicated by the asterisks.

long (Fig.1 and 4). All the hybridizing bands correspond to each of the class II β chain genes reported in the accompanying paper (Kawai, 1990) and other reports. Thus, the EcoRI 20kb band corresponded to the DRβIII gene, 9.0kb to DPβ1 and DPβ2 genes (Ando et al., 1986), 6.0kb to DRβI and isolated β1 exon next to the DRα gene, 5.0kb to DRβI, βII

and βIII genes, 4.0kb to DRβI and isolated leader sequence downstream from DRβIII gene, and 3.4kb to DRβIII gene (see Kawai, 1990). Clone analysis suggested that probes of DRβ and DQα cDNAs would show RFLPs, but polymorphic bands were hidden by other nonpolymorphic bands. For example, 3.4kb EcoRI of PGF (Dw2) and 4.2kb EcoRI fragment of AKIBA (Dw12) expected to show polymorphism by use of the DRβ 5'UT specific probe were hidden by nonpolymorphic bands derived from DRβII and DRβI genes, respectively. Similarly the DQα cDNA probe also gave less polymorphisms and every hybridizing bands observed could correspond to that of the DQα and the DXα genes. In contrast, the DQβ cDNA probe generated highly polymorphic blot patterns using each restriction enzyme, EcoRI, PstI, MspI and TaqI (Fig.3), suggesting the ability of the Dw typing. The restriction maps of cosmid clones encoding HLA class II genes suggested that all of the polymorphic bands except of EcoRI 10kb band of AKIBA which seemed to encode the DPβ1 gene (Ando et al., 1986), were derived from the DQβ gene. Hybridizing patterns that 9.6kb and 2.6kb bands were seen with EcoRI digestion in Dw2, as well as 18kb band in Dw12, was coincident with the results of the DQ cosmid clones (Kawai, 1990). Eleven polymorphic bands selected for Dw typing in DR2 as follows; EcoRI 18kb, EcoRI 2.6kb, PstI 6.5kb, PstI 4.6kb, PstI 1.4kb, MspI 5.0kb, MspI 0.4kb, TaqI 7.0kb, TaqI 5.4kb, and TaqI 0.7kb.

Dw typing of IDDM patients and healthy controls

Serological studies showed that the frequencies of the specificities DR3 and DR4 are increased in IDDM patients as compared to normal controls, while the frequency of DR2 is decreased, suggested relevant susceptibility–or resistance–related factors are involved in DR2 haplotypes (Svejgaard et al., 1984; Bertrams and Baur 1984). The results of statistical analysis of DR frequencies in IDDM patients were shown in Table 2, clearly indicating the remarkable decrease of DR2–positive IDDM patients ($\chi^2=162$). I tried to investigate the genetical bases of the resistance of DR2 against IDDM. Genomic DNAs from fifteen IDDM patients' and fourteen healthy controls' peripheral blood lymphocytes with DR2 specificity which was determined at the Department of Transplantation, School of Medicine, Tokai university, were digested with four different restriction enzymes (EcoRI, PstI, TaqI and MspI) and probed with the DQβ cDNA. Autoradiograms using EcoRI and MspI as restriction enzyme were shown in Fig.5. Neither specific band nor unexpected band only for the IDDM patient in comparison with the result of homozygous typing cell lines was found, suggesting that IDDM patients might have no specific fragment observed by Southern blot. Dw typing panel were shown in Fig.6. Twenty six out of twenty nine Dw subtypes derived from DR2 of the IDDM patients and healthy controls could be

Table 2. HLA–DR haplotypes in Japanese IDDM patients and healthy controls*.

	DR2	not DR2	
IDDM	41	394	
healthy	162	310	$\chi^2=162$

* HLA–DR haplotypes were serologically determined at the department of plantation, school of medicine, Tokai Univ.

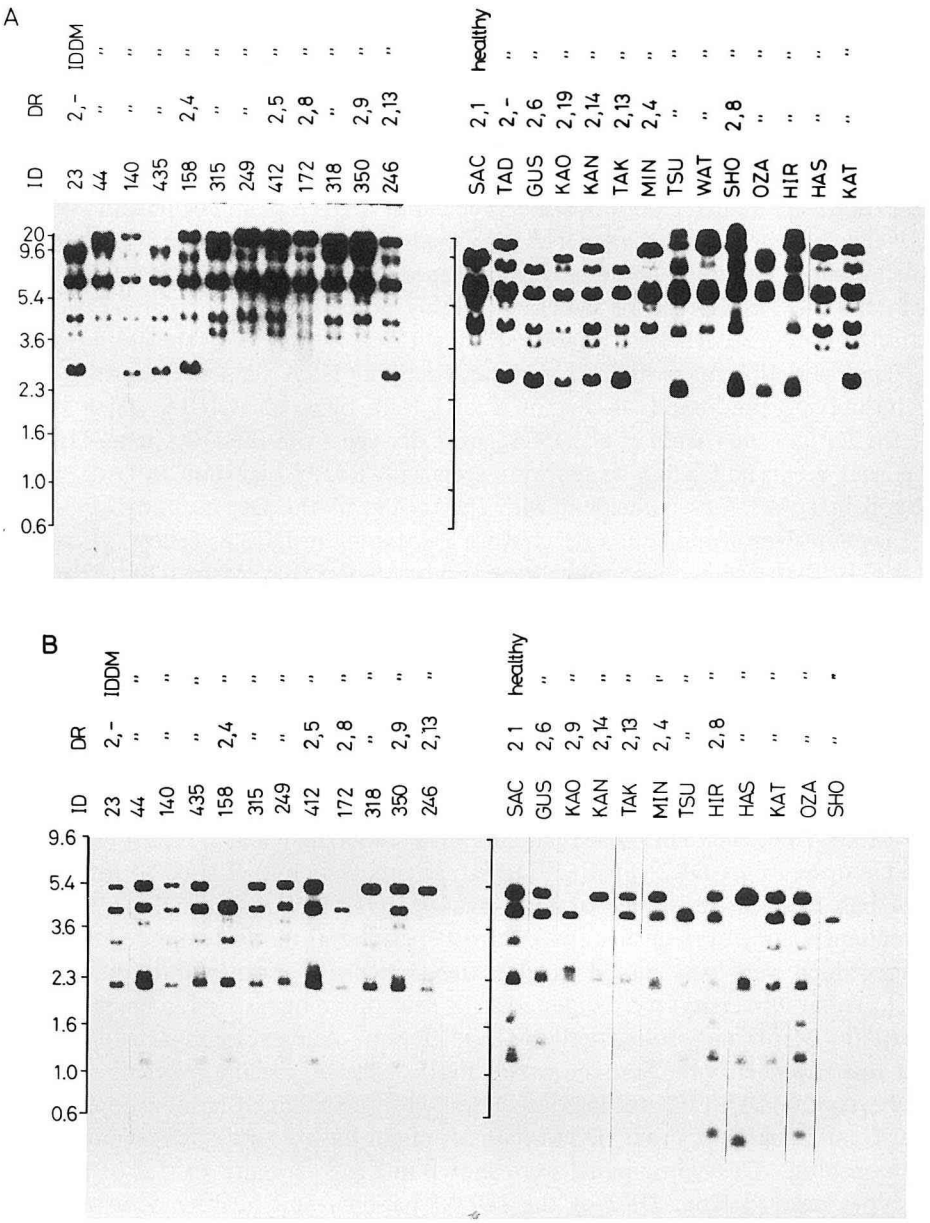

Fig. 5. Autoradiograms of blot hybridization analysis of DR2 positive IDDM patients and healthy controls using the DQβ cDNA as probe. DNA from peripheral bleed lymphocytes were digested with EcoRI(A) and MspI(B). Individual DR haplotypes were listed at the top.

determined clearly. For example, IDDM patient, #23, has six informative bands as follows, P1.4, P4.6, T5.4, E2.6, P6.5 and M5.0. First three bands (P1.4, P4.6, T5.4) were Dw21 specific and last four bands (T5.4, E2.6, P6.5, M5.0) were specific for Dw2, and no additional band specific for Dw12 was observed, clearly suggesting that he had Dw2/Dw21 specificities. Haplotypes of three persons, #412, #172 and KAT, were undetermined, because that serological DR typing might be mistaken or new Dw haplotype presently

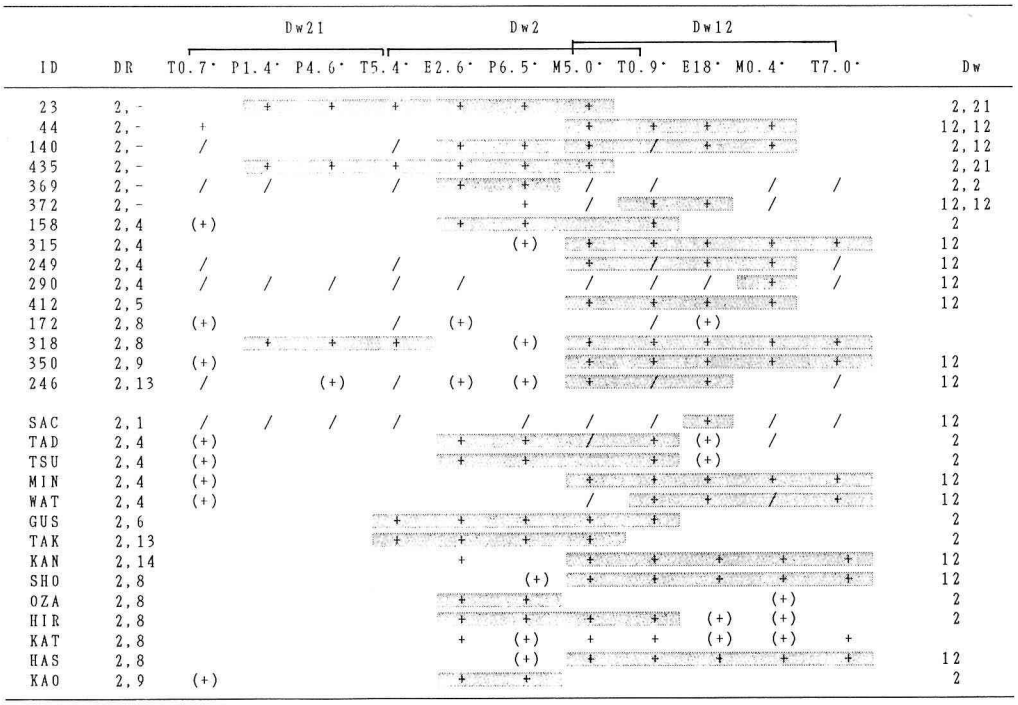

Fig. 6. Diagrammatic patterns of DQβ DNA fragment length of IDDM patients and healthy controls. Dw types and their polymorphic fragments are listed at the top. E, P, T and M represent the restriction endonucleases, EcoRI, PstI, TaqI and MspI, respectively and the numbers indicate length of hybridizing bands in kb. Symbols as follows; +, positive; /, unable to read or not done. Bands seemed to be derived from other haplotype than DR2 (Maeda et al., 1989) were shown by (+).

Table 3. Dw type frequency in DR2 IDDM patients and healthy controls.

		Dw2	Dw12	Dw21	others
IDDM	(N=15)*	6	11	2	2
control	(N=14)	7	6	0	1

* Number of investigated individuals.

unknown. Table 3 showed Dw type frequencies in DR2 positive IDDM patients and healthy controls. Although frequency of Dw12 and Dw21 in IDDM patients was apparently increased in comparison of that of healthy controls, no statistical significance was observed. Frequency of Dw subtype Dw21 in DR2 is reported to be significantly increased in Caucasian (Bohme et al., 1986; Segall, 1986, Sorretino et al., 1985). The subtype Dw21 accounts for only about 6% of normal DR2 haplotypes, 77% of IDDM DR2 chromosomes, however, are Dw21. In the present study, I analyzed samples of 17 DR2 IDDM patients and 14 DR2 healthy individuals in Japanese. All of Dw haplotypes except three persons were clearly determined by Southern blot analysis using the DQβ cDNA probe and no statistical significance of Dw frequencies was observed (Table 3). It is, however, interesting that only two IDDM patients, but no healthy individual, have Dw21

specificities. Some reports (Todd et. al. 1987, 1988, Morel et. al. 1988) suggested that IDDM susceptibility is largely dependent on the identity of amino-acid residue 57 of the DQβ chain. Thus, amino acid, aspartic acid (Asp) at position 57 in the DQβ chain is negatively associated with this disease. DQβ chain of Dw2 and Dw12 have Asp at position 57, on the other hand, Dw21 has a residue, serine. Recently, however, single amino-acid, aspartic acid at position 57, is not sufficient for development of insulitis, but that the amino acid at position 57 of the β chain is involved in the interaction between MHC class II molecules and the T-cell receptor of the autoreactive T cells in the transgenic NOD mice (Miyazaki et al., 1990; Slattery et al., 1990; Lund et al., 1990). This implies that conformation of the DQ molecule is involved in the IDDM development. No significant increase of the frequency of Dw21 in Japanese patients might be resulted from more participation of three-dimensional conformation of the DQ gene antigen in IDDM than single amino acid at position 57. The DQ transfectant reported accompanying paper (Kawai, 1990) remains valuable for precise analysis of the mode of the DQ antigen in the disease process.

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著者： 河合 純，〒553 大阪市福島区鷺州5-12-4，塩野義製薬株式会社研究所