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

I. Isolation and Characterization of the Genomic Clones of the Class II Antigens from DR2DQw1 Haplotypes*

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Abstract 440kb length of overlapping cosmid clones, including DO, DX/DV, DQ and DR regions, was obtained from two DR2DQw1 haplotypes, AKIBA and PGF, and the gene structures were investigated. Differences of the restriction sites between AKIBA (Dw12) and PGF(Dw2) were most concentrated near the DQβ region, than any other class II genes. Deletion or insertion of about 5kb long region was observed upstream from the DQβ gene. DR region contained three β, one α, and two isolated β chain exon fragments. The DQβ, DRβI and DRβIII genes were expressed, together with their pairing α chains, after transfection into mouse L cells. Restriction enzyme mapping of the DRβ clones and reactivity of their products against monoclonal antibodies showed that the DRβI and DRβIII genes encoded the nonpolymorphic and polymorphic DRβ chain, respectively. This arrangement is the reverse of that observed in other haplotypes. The alignment of the HLA class II genes on the chromosome 6, however, was consistent with other haplotypes. These results suggest that the susceptibility to mutations or gene conversions responsible for genetic polymorphisms depends on the gene itself and not on its location. Furthermore, absorption experiments of anti-DR2 allosera by the DRα/DRβ transfectants revealed that the so-called DR2 specificities were determined by multiple epitopes.

INTRODUCTION

Human chromosome 6 contains a cluster of genes, known as the major histocompatibility complex (MHC), HLA, that encode a number of cell surface glycoproteins involved in various immune interactions (Kaufman et al., 1984). The HLA-A,-B and-C are designated class I antigens, and HLA-DR,-DQ, and-DP antigens are designated class II antigens. Whereas the class I antigens are expressed on almost all types of cells, the class II antigens have limited tissue distribution and are expressed on antigen presenting cells, such as macrophages and dendritic cells. The class II antigens define determinants involved in central events of immunoregulation, such as stimulation of appropriate T cells in a specific manner(Nagy et al., 1981). The class II antigens are heterodimers of two glycosylated, membrane-integrated chains, α and β. The polymorphism of these molecules has been extensively studied using serological and cellular techniques (Termijtelen et al., 1982), two-dimensional polyacrylamide gel

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electrophoresis (Nepom and Holbeck, 1984a), and molecular biological techniques at the genomic level (D. Cohen et al., 1984). Polymorphic determinants of the HLA class II antigens reside mainly in the β-chain (Charron and McDevitt, 1979; Silver and Ferrone, 1979), although at least one class of α-chains, DQα, also displays genetic variability (Auffray et al., 1983; Chang et al., 1983; Schenning et al., 1984).

An analysis and a comparison of the HLA class II region among various haplotypes is interested because many diseases are associated with HLA class II haplotypes (Svejgaard, 1982), such as narcolepsy (Honda, 1984), myasthenia gravis (Keller, 1987) and insulin dependent diabetes mellitus (Todd, 1988). They have found that linkage disequilibrium between the various alleles of the different loci from DR to DX genes. Narcolepsy is strongly associated with DR2Dw2 (Honda et al., 1984; Inoko et al., 1986), especially linked restriction fragment length polymorphisms of DQβ gene (Inoko et al., 1986). Todd et al. (1990) reports suggested the association of insulin dependent diabetes mellitus with DQ gene structure. These disease associations suggest that some genetic background which affect the development of these diseases locate in the HLA class II region.

The HLA class II gene region encodes at least three expressed products, HLA-DP, DQ and DR with two different DR products being expressed in most haplotypes(Trowsdale et al., 1985). A genetic approach, however, proved evidence for several other α and β genes. There are cloned six genes involved in the class II genes, DP, DO, DX, DV, DQ, and DR. Some of the class II genes, particularly in the DR and DQ regions, are in strong linkage disequilibrium. Therefore, it is quite difficult to dissect out their individual functions. Although numbers of genes involved in each subregion is constant, that of the DRβ genes vary in various haplotypes. There are usually three or four DRβ genes, named DRβI, DRβII, DRβIII, and DRβIV in the centromere to telomere direction (Auffray et al., 1985; Sorretino et al., 1985; Böhme et al., 1985). One or two of the DRβ genes are pseudogenes(Spies et al., 1985; Rollini et al., 1985, 1987; Larhammar et al., 1985;). In the DR3 and DR4 haplotypes the DRβI gene encodes the polymorphic serological specificity (DR3 or DR4) and the DRβIII or DRβIV gene encodes the supertypic or nonpolymorphic one (DRw52 or DRw53)(Spies et al., 1985; Rollini et al., 1985). Two DRβ genes are also known to be expressed in the DR2 haplotype, leading to the formation of two DR products, on the basis of biochemical and cDNA sequencing studies(Lee et al., 1987; Nakatsuji et al., 1986; Nepom et al., 1984b; Wu et al., 1987). One of these expressed genes encodes a relatively nonpolymorphic protein which is believed to correspond to the supertypic DR allospecificity in other haplotypes such as DRw52 and DRw53, in DR3 or DR4 haplotypes, respectively. In the DR2 serotype such a supertypic antigen has not yet been serologically defined. The other encodes a protein that is polymorphic among the Dw2, Dw12 and Dw21 subtypes of DR2, and is believed to correspond to the DRβI locus-encoding molecule.

In this study we have cloned DO, DX, DV, DQ, and DR genes from the two series of genomic gene libraries on overlapping cosmid clones, in order to analyze and compare their gene structure among different Dw haplotype, Dw2 and Dw12, but the same DR2DQw1 haplotype. Furthermore, DQβ and two DRβ genes were transfected separately, together with their pairing α chain gene, into mouse L cells. DR antigen determinants were also investigated by absorption of haplotype specific sera with DR antigen-expressing transfectants.

MATERIALS AND METHODS

Isolation and characterization of genomic clones

Genomic gene libraries were constructed from the two consanguineous homozygous B lymphoid cell lines (LCL), PGF and AKIBA whose haplotypes are DR2,Dw2,DQw1,DPw4 and DR2,Dw12,DQw1,Cp63, respectively. Briefly, DNAs from LCLs were partially digested with Sau3AI and fractionated on sucrose gradients. Fractions containing DNA fragments 35–45 kilobases (kb) long were collected, ligated with the arms of the cosmid vector, pJB8 (Ish-Horowicz and Burke, 1981) and transfected into *Escherichia coli* 1046 using *in vitro* packaging extracts. A total of 6,900,000 cosmid colonies were spread, replicated, and screened by colony filter hybridization, as described by Maniatis et al.(Maniatis et al., 1982). Single colonies containing desired HLA-class II cosmids were isolated through two or three times hybridization. Libraries were screened with cDNA and genomic DNA fragments labeled with [^{32}P]CTP. Colony hybridization was carried out in 5x SSPE (1x SSPE is 0.15M NaCl/ 10mM NaH_2PO_4/ 1mM EDTA)/ 5x Denhardt's solution (1x Denhardt's solution is 0.02% bovine serum albumin/ 0.02% ficoll/ 0.02% polyvinylpirrolidone)/ 50% formamide/ 0.1% SDS/ sonicated salmon sperm DNA (100μl/ml) for 16hr at 37°C. Filters were washed in 3x SSC(1x SSC is 0.15M NaCl/ 0.015M NaCl/ Cit)/ 0.1% SDS/ at room temperature for 5 min, 2x SSC/ 0.1% SDS at room temperature for 5 min, 1x SSC/ 0.1% SDS at room temperature for 5 min, 1x SSC/ 0.1% SDS at 65°C for 15 min, and 0.2x SSC/ 0.1% SDS at 65°C for 15 min, and were exposed to Kodak XRP-5 films. Clones hybridizing to the specific probes were grown and their DNAs were prepared by the miniprep method. Isolated cosmid clones were mapped by standard single and double digestions with restriction endonucleases followed by Southern blot hybridization using specific cDNA fragments, under stringent conditions. Cosmid clones designated pAK or pA, and pP are derived from the libraries of AKIBA and PGF cells, respectively.

Probes

The probes used for screening and characterization of class II genes were isolated by agarose or polyacrylamide gel electrophoresis after restriction enzyme digestion, electroeluted into dialysis tubes, and radioactively labeled with [^{32}P]dCTP by random primed DNA labeling kit (Boehringer). The fragments used are as follows (see Fig.1). The probes for the DRβ gene were the 160bp PstI-SacI and 290bp PstI fragments and the entire sequence of the DRβ cDNA clone, pDRβ102 (Inoko et al., 1985b) which encode the 5' untranslated (5'UT) region and the 3' untranslated (3'UT) region, respectively. The 220bp PstI fragment of the DRβ cDNA clone, pDRβ101 (Inoko et al., 1985b), a part of the β_1 domain; The 700bp PstI fragment and the entire sequence from the DRα cDNA clone, pDRH2 (Lee et al., 1982), was used, which encodes a part of the α_2 domain, the whole transmembrane-cytoplasmic region (TM/CY) and the 3'UT region. 1220bp DQβ cDNA, pDCβ101, all of the coding sequence for DQβ gene (Inoko et al., 1985b). The 300bp PstI-DdeI fragment, which encode a part of α_2, TM, CY and 3'UT, and the entire 980bp of the DQα cDNA, pDCα107, respectively. The 490bp DOα cDNA, pDOα20, a part of α_2 domain, TM, CY AND 3'UT region. The 2.0kb PstI fragment of the DVβ genomic clone, λHMCβ19, which encode a part of DVβ_2 domain. The 2.7kb EcoRI fragment of pAKQ056, which locate 18kb upstream from the DQβ gene (see Fig.5).

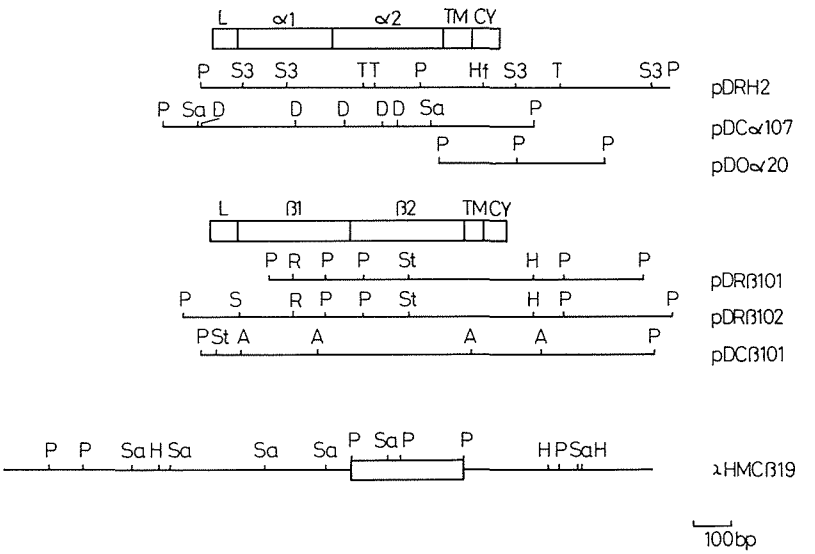

Fig. 1. Restriction maps of cDNA clones for HLA class II antigens. L, leader sequence; α_1, α_2, β_1 and β_2, regions coding for first and second extracelluar domain of α and β chains; TM, transmembrane region; CY, intracytoplasmic region. Abbreviations for restriction enzymes are follows, H, HindIII; P, PstI; St, StuI; Sa, SacI; A, AvaI R, RsaI. Open box in genomic clone, λHMCβ19 represents the exon encoding the DVβ gene.

Genomic Southern hybridization

EB transformed LCLs and PBLs (1–2x10^7 cells) were lysed at 50°C for 16hr in 50mM Tris HCl (pH8.0), 20mM EDTA, 100mM NaCl, 1% SDS, and 150μl/ml proteinase K. Protein was removed from high molecular weight DNA by 400mM Tris saturated phenol and the DNA was precipitated in the presence of 0.3M K–acetate with 2 volumes of cold 100% ethanol. The DNA precipitated was dissolved in H_2O at a concentration of 1μg/μl. Four different restriction endonucleases, EcoRI, PstI, TaqI, and MspI (Toyobo Co., LTD.), were used for digestion. Samples of restriction enzyme cleaved genomic DNA (7.0μg) were subjected to electrophoresis in 0.7% agarose and transferred to BioTrace membranes (Gelman Sciences Inc.) as described by Southern (Southern, 1975). After transfer, the filters were prehybridized (50% formamide, 5x Denhardt's solution, 5x SSPE, 1% SDS, 5% Dextran sulfate, 100μg/ml sonicated salmon sperm DNA) at 42°C for 16hr. Radioactively labeled cDNA fragments were added at a concentration of 1x10^6 cpm/ml to the hybridization mixture and incubated with the filters at 42°C for 16hr. The filters were washed with 2x SSPE at room temperature for 5min, with 2x SSPE/ 0.5% SDS for 15min, at 65°C and with 0.5x SSPE/ 0.5% SDS for 15min at 65°C. The filters were then exposed to Kodak XRP–5 films with an intensifying screen at –70°C.

Transfection of HLA class II genes into mouse L cells

The cosmid clones encoding HLA class II α and β genes were cotransfected with the plasmid, pSV2neo, containing the neo gene, into mouse L cells using the modified calcium phosphate technique (Chen and Okayama, 1987) to obtain cell lines expressing HLA class II antigen on their cell surfaces. The transfected cosmids are as follows, pAKQ4116 and

pAKQ056, AKIBA DQα/β genes; pPQ364 and pPQ362, PGF DQα/β genes; pPR541 and pAKR4643, DRα/βI genes; pPR541 and pPR241, DRα/βIII genes (see Fig.5 and 8). Transfectants were grown in Dulbecco's modified eagle medium supplemented with 10% fetal calf serum, penicillin and streptomycin, and selected using 400μg/ml Geneticin (Gibco, Grand Island, New York). Geneticin-resistant colonies resulting from each transfection were cloned, stained with the anti-DQ MoAb, Leu10 (Becton Dickinson) and the anti-DR MoAb, L243 (Becton Dickinson) for flow microfluorimetric analysis and further cloned by limiting dilution.

Flow microfluorimetric analysis

1x10^6 cells were washed with cold PBS and incubated with 30μl of antibody at the concentration enough to bind LCLs (data not shown) for 30 min on ice. After three more washes in PBS, the cells were resuspended in 50μl of fluorescene isothiocyanate (FITC) conjugated goat anti-mouse immunoglobulin (Cappel) and incubated for a further 30 min at room temperature. Following three final washes, the cells were analyzed on the FACS. The specificities of the MoAbs used are as follows, L243,HLA-DR; Hu30, HLA-DR1 and DR2 (provided by Dr. Aizawa) (Nishimura et al., 1984); NDS15.38, HLA-DR2 (Makgoba et al., 1983); AB8.25, DR2+5+shortDR3 (Colombani et al., 1983) (distributed for the Tenth International Histocompatibility Workshop studies:10th IHWS); Leu10, HLA-DQ; B7/21, HLA-DP (Becton Dickinson).

Alloantisera absorption assay by DR gene-transfected L cells

The specificities of alloantisera used were as follows, 1030, 1026 and 1029;DR2 (these three were selected from the best alloantisera submitted to the 10th IHWS); Ex107, DR4; Ex115, DR1,2,4 and 8; Ex99,DR1 and DR2; JR21-224,DR2. The specificities of the last four alloantisera were determined at the department of Tokai Univ. school of medicine. For absorption, alloantisera were incubated (30 min at 37°C and 90 min at 4°C) with 7x10^6 cells prior to the test. 3x10^3 LCLs or 5x10^3 B cell-enriched fraction from human peripheral blood lymphocytes (PBLs) were used for cytotoxicity assay. Briefly, cells were incubated for 1hr at 37°C with absorbed or unabsorbed alloantisera(1μl), and followed by incubation with 5μl rabbit complement for 2hr at room temperature. The percentage of dead cells was scored after eosin staining.

RESULTS

Cloning of class II genes

Two set of cosmid gene libraries were constructed from 30-45kb long high molecular weight genomic DNA of the consanguineous HLA-homozygous lymphoid cell lines, AKIBA (DR2, Dw12, DQw1) and PGF (DR2, Dw2, DQw1). By screening these libraries with HLA class II cDNA clones as probes, a set of cosmid clones for DOα, DXα, DXβ, DVβ, DQα, DRβ, and DRα genes were isolated. Cosmid screening was performed through two steps (Fig.2). 30,000 colonies were spread on each nitrocellulose filter, replicated and screened by HLA class II probes (primary screening). The positive clones were picked up, spread on small filters and hybridized with the same probe (secondary screening), resulting single colonies were isolated. Colony hybridization was done under the stringent condition to suppress cross hybridization to other subregion genes.

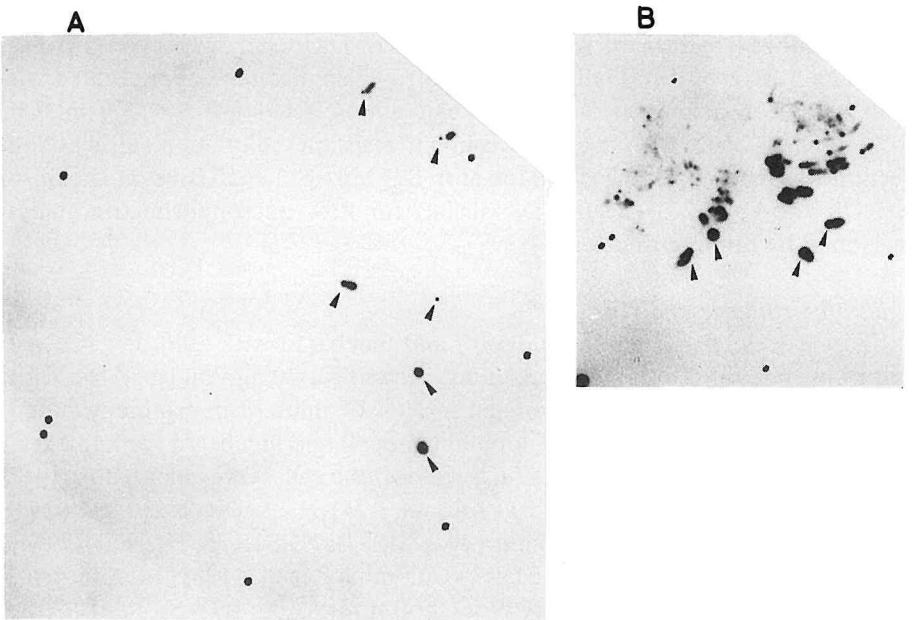

Fig. 2. Autoradiograms of colony hybridization. The replicated filters were hybridized with DOα cDNA, pDOα20. Arrows indicate a part of positive colonies. Filters were oriented by placing a series of ink marks on their edges. (A) Primary screening of AKIBA, (B) Secondary screening; Two picked up colonies from the master plate were spread on the right and left of the nitrocellulose filter separately.

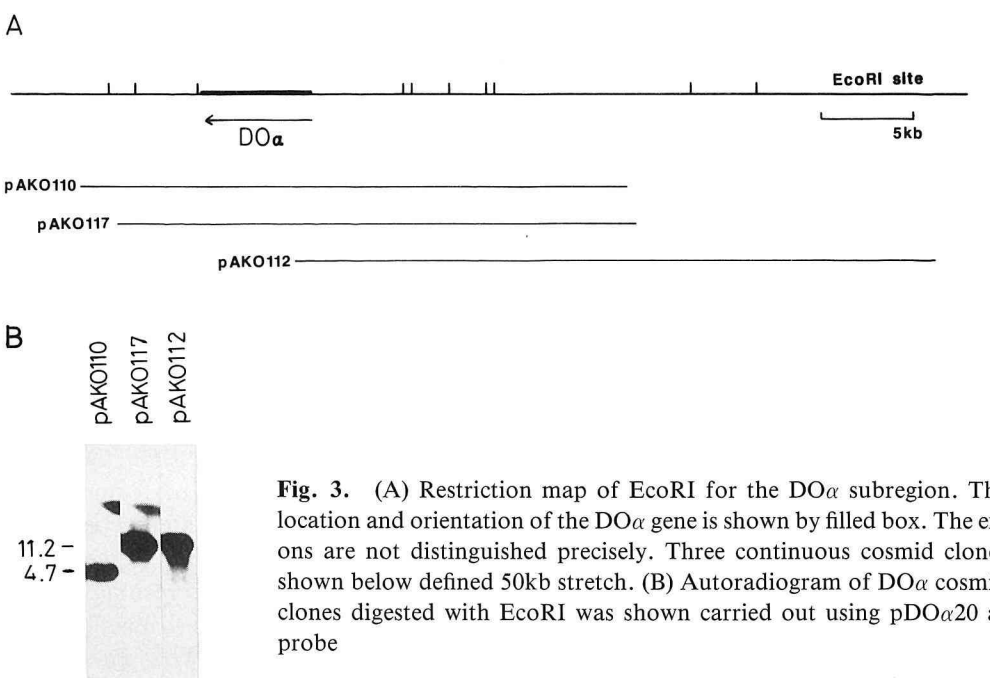

Fig. 3. (A) Restriction map of EcoRI for the DOα subregion. The location and orientation of the DOα gene is shown by filled box. The exons are not distinguished precisely. Three continuous cosmid clones shown below defined 50kb stretch. (B) Autoradiogram of DOα cosmid clones digested with EcoRI was shown carried out using pDOα20 as probe

Isolation and analysis of the DOα, DQ, and DX/DV genes

Three continuous cosmid clones representing a length of about 40kb in the DOα subregion, identifying one DOα gene, were obtained from AKIBA by colony hybridization with DOα cDNA clone, pDOα20 (Inoko et al., 1985a), as a probe. Restriction map of DOα cosmid clones and the autoradiogram using DOα cDNA probe were shown in Fig.3. Restriction map of about 20kb region from DOα were identical to that of the previous report (Inoko et al., 1985a).

DX genes have high homology to the DQ genes at nucleotide sequence (Auffray et al., 1984, 1987; Okada et al., 1985; Jonsson et al., 1987), resulting successful isolation of the DX genes from AKIBA using the DQα and βcDNA as probe. Southern blotting probed by DQβ cDNA were shown (Fig,4B).

DVβ is a class II light chain pseudogene, found by Ando et. al. (1986a), which has highest homology to the DQβ gene among class II β genes. Two cosmid clones, pA412 and pPKV5123–2, were obtained from AKIBA and PGF, respectively, using 2.0kb PstI fragment of λHMCβ19 encoding the DVβ gene. High homology of the DVβ with DQβ gene was confirmed by Southern blot analysis of the DVβ clones isolated here (Fig.4C). A comparison of the restriction map between pA412 and pAX523, encoding the DVβ and the DXα region, respectively, suggested that these two clones overlapped about 8kb long. Southern blot analysis using a short fragment in the overlapping region as probe confirmed that pA412 and pAX523 shared the same locus (Data not shown), indicating that the DVβ gene located 15kb upstream from the DXα gene (Fig.4A).

Isolation of the DQα and β genes

At first, I expected to obtain DQα cosmid clones using entire DQα cDNA (pDCα107) as probe, I obtained, however, no DQα cosmid clone, but many DPα and DXα genes from

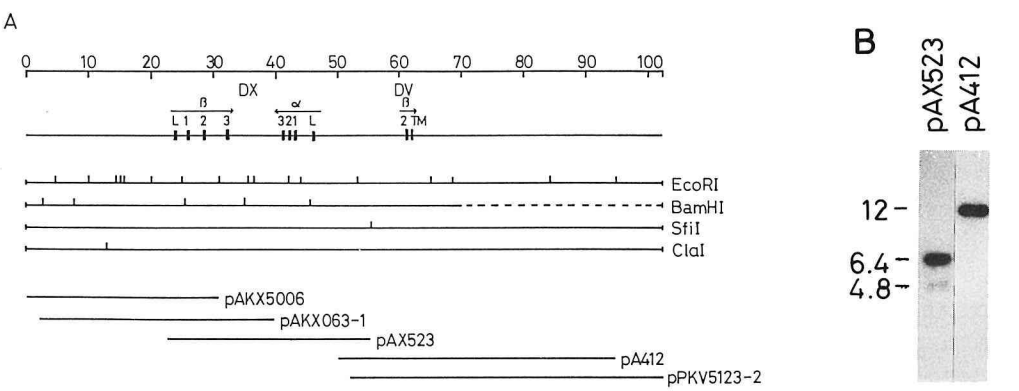

Fig. 4. An organization and restriction map of the DX–DV subregion is shown (A). The top line gives the scale in kb. The 100kb stretches of DNA are defined by overlapping cosmid clones shown below the cleavage sites of the restriction enzymes, EcoRI, BamHI, SfiI, ClaI. Down stream region from DVβ gene is not yet mapped with BamHI (dotted line). The exons are shown by black boxes. L; leader, 1, 2; first and second external domains; TM; transmembrane region; 3; 3' untranslated region of the mRNA. Arrows above the map indicated the direction of transcription of each gene. Southern blot of the DXβ and DVβ cosmid clones, pAX523 (B) and pAK412 (C), respectively, digested with EcoRI(E) and/or PstI(P) hybridized with DQβ cDNA, pDCβ101.

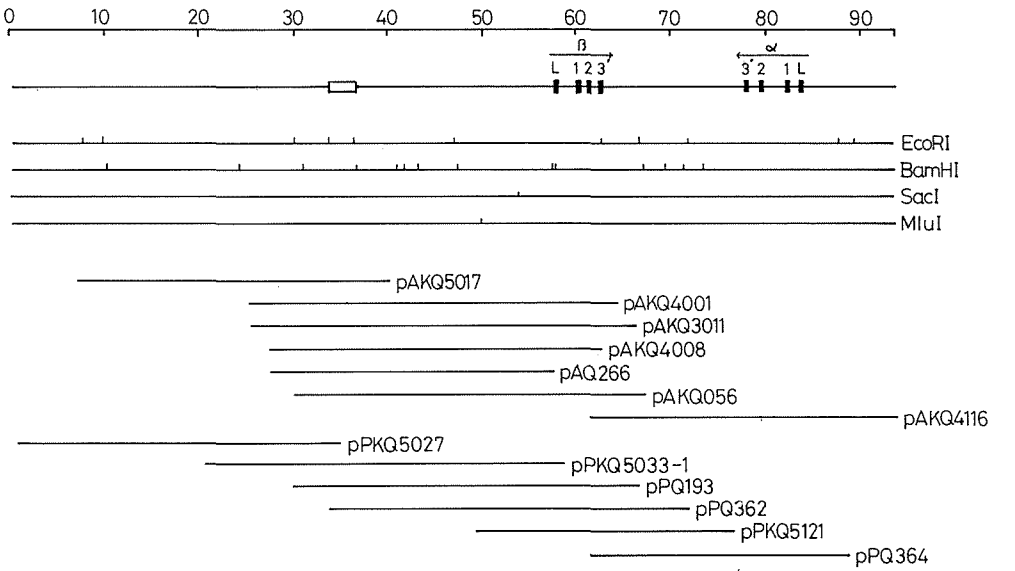

Fig. 5. A organization and restriction map of the DQ subregion including one α and one β genes. See a legend of Fig.4 for abbreviations. The open box 20kb upstream from the DQβ gene indicates the 2.7kb EcoRI region of pAKQ056 used as the walking probe. The cleavage sites of the restriction enzymes were shown for AKIBA.

the libraries of AKIBA because of cross hybridization. Similarly in the case of screening of the DQβ gene using entire DQβ cDNA as probe, much more DPβ and DXβ genes were isolated than DQβ cosmid clones. Genomic DNA sequences including the DQα and β genes might inhibit the growth of the host E. coli resulting in low number of clones containing DQ α and β genes. Then I chose 300bp PstI–DdeI fragment of pDCα107 as probe because of low homology in 3'UT region between DQ and other genes (Auffray et al., 1987) (see Fig.1), resulting that a cosmid clone, pAKQ4116, including DQα gene, was obtained (Fig.5).

Several clones for the DQβ gene were isolated. Moreover, I tried to isolate clones covering upstream region, or intervening region between DQβ and DVβ, by chromosomal walking method. Two clones, pAKQ5017 and pPKQ5027–1 were obtained from AKIBA and PGF respectively using 2.7kb EcoRI fragment of pAKQ056 about 25kb upstream from the DQβ gene (Fig.5).

In all a region of about 100kb in length including DQα and β genes were cloned. DQ gene region restriction maps of AKIBA (DR2,DQw1,Dw12) and PGF (DR2,DQw1,Dw2) were like each other, however, some differences were observed (Fig.6) and those polymorphisms concentrated near the DQβ gene. It suggests a possibility to determine the Dw specificities by investigation of the structure of DQβ region. It is described in my accompanying paper (Kawai, 1991).

The restriction maps of the region about 50kb upstream from the DQβ gene of AKIBA were remarkably differed from that of PGF suggested a dynamic change of gene structure among various Dw types in this region. Then, two fragments from the cosmid clones derived from AKIBA were used as probes to analyze gene structure upstream from

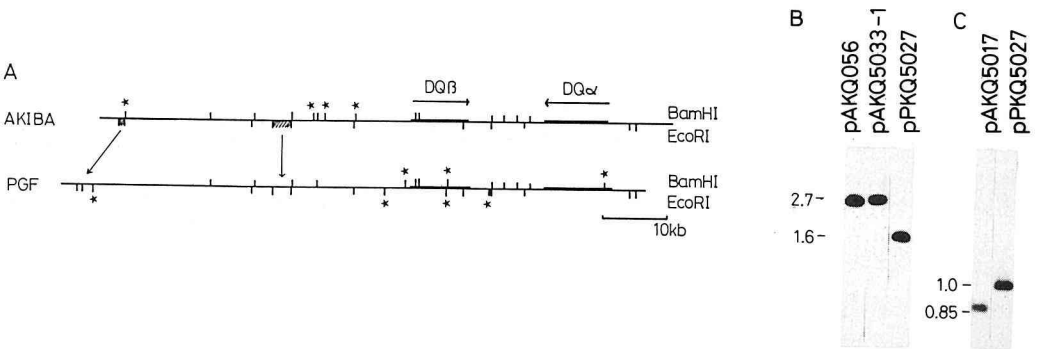

Fig. 6. (A) Differences of the DQ subregion restriction sites between DR2Dw12(AKIBA) and DR2Dw2(PGF). Filled boxed indicate the DQα and β genes. different EcoRI and BamHI sites between Dw2 and Dw12 were signified by asterisks. Hatched fragment, 0.85kb and 2.7kb EcoRI fragment, were used as site specific probe, and their hybridizing regions were showed by arrows. Southern blot of the DQβ upstream cosmid clones digested with EcoRI using 2.7kb and 0.85kb EcoRI fragments of pAKQ5017 as probe (B and C, respectively).

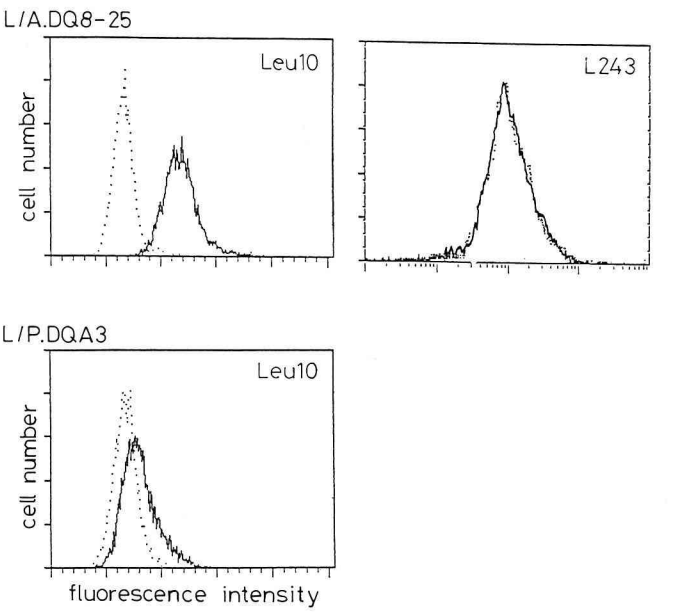

Fig. 7. Flow microfluorimetric analysis of the transfectant, L/A.DQ8–25 and L/P.DQA.3 which express DQ antigens, using anti-HLA class II MoAbs. The specificities are: L243, HLA-DR; and Leu10, HLA-DQ. 5,000 cells were analyzed in each profile and plotted as log. fluorescence intensity against cell number by solid lines. In each profile, the negative control is the transfectant cell line stained with FITC rabbit anti-mouse Ig alone shown by dotted lines.

DQβ gene. Although 2.7kb EcoRI fragment of pAKQ5017 hybridized to the 1.6kb EcoRI fragment of pAKQ5027 located on the same position as AKIBA, 0.85kb EcoRI fragment of pAKQ5017 hybridized to 1.0KB EcoRI fragment of pPKQ5027 located about 5kb upstream from the corresponding position in AKIBA (Fig.6B), suggested insertion or

deletion of 5kb length might have occurred. This deletion/insertion event suggested other many deletion/insertion events in HLA class II gene region. It might explain the different distance between DV and DQ genes (Inoko et al., 1988)

I also confirmed that cosmid clones included the DQ gene by mAb reactivity against cell surface products on DQ cosmid-transfectants. Two clones of the transfectants designated, L/A.DQ8-25 and L/P.DQA.3, were obtained. L/A.DQ8-25 was transfected with the AKIBA DQα (pAKQ4116) and DQβ (pAKQ056) genes and L/P.DQA.3 with the PGF DQα (pPQ364) and DQβ (pPQ362) genes. Analysis of the L/A.DQ8-25 and L/P.DQA.3 transfectants using flow microfluorimetry and anti-HLA class II specific MoAbs clearly indicated expression of the DQ gene products on the cell surface. They reacted with the anti-DQ MoAb, Leu10 but not with anti-DR MoAbs, L243 and Hu30(Data not shown), confirming that the cosmid clones cloned here encode the DQ gene (Fig.7). It is not clear that weak binding of Leu10 against L/P.DQA.3 transfectant is due to low amount or aberrant conformation of expressed DQ antigen. it was reported that the transformant class II molecules differed from "normal" class II molecules in their carbohydrate moiety (Tanigaki et al., 1987; Tosi et al., 1986).

Structure of the DR subregion

Two sets of cosmid clones including three DRβ genes spanning about 140kb, and one DRα gene spanning about 70kb were isolated. They were mapped using exon-specific cDNA fragment probes, although the TM/CY exons could not be distinguished precisely (Fig.8). Three β genes were designated βI, βII and βIII, following their orientations of transcription. An additional region homologous to the DRβ leader sequence was found about 50kb downstream from the βIII gene. Spies et al. (1985) also reported that the genetic region between the DRβ and DRα genes contained four repeats of the DRβ leader sequence in DR4 haplotype.

One DRα gene was cloned (Fig.8) and the β_1 domain exon downstream from the DRα gene described previously (Meunier et al., 1986) was also identified at the same position in the cosmid, pPR541. The restriction map near the DRα gene in the DR2 haplotype was almost identical to those of other haplotypes (Spies et al., 1985; Meunier et a;l, 1986; Das et al., 1983), although in the 5' upstream flanking region it differed remarkably from others. Homology of the restriction map in the DRα gene region among the haplotypes seemed to be confined to about 30kb around the DRα gene.

In the course of this restriction mapping, the DRβII gene failed to hybridize to the probe specific for the 5'UT region of the DRβ cDNA even under less stringent conditions (Fig.9), suggesting that it might have been a pseudogene. Additionally, no transfectant was obtained expressing a product detected by anti-HLA-DR MoAb, L243, after the DRβII and the DRα gene-cotransfection to mouse L cells (Data not shown). This structure of the DRβII gene differed from those of other haplotypes, DR3 and DR4 in that it contained all of the exon except for the first (Spies et al., 1985; Rollini et al., 1985, 1987; Larhammar et al., 1985). In contrast to the DRβII gene, both the DRβI and the DRβIII genes contained the 5'UT, β_1, β_2 and 3'UT exon necessary to express the HLA class II antigen, implying that these two DRβ genes should be expressed, which was confirmed by transfection experiments as described later.

The number of HLA DRβ genes

Although every DR haplotype (DR1–9) has one DRα gene, DRβ genes were reported to vary in number between different DR specificities. Böhme et al. (1985) described three DRβ genes in DR2, 3, 4, 6 and 7, two genes in DR1, one gene in DR8 and two or three genes in DR5 and 9. Andersson et al. (1987) obtained four DRβ genes in genomic clones from the haplotype, DR4Dw4. Additional isolated exon for the DRβ chain observed between the DRα and DRβ genes (Andersson et al., 1987; Larhammar et al., 1985; Spies et al., 1985; Rollini et al 1985; Meunier et al., 1986). Then, Southern blotting of genomic DNA extracted from AKIBA and PGF were carried out using each DRβ domain specific probe under the stringent condition to estimate the number of the DRβ gene exon in DR2 (Fig.10). High molecular weight DNA of 7μg after digestion with restriction enzymes, EcoRI, BamHI and PstI, were electrophoresed, transferred to nylon membranes, and

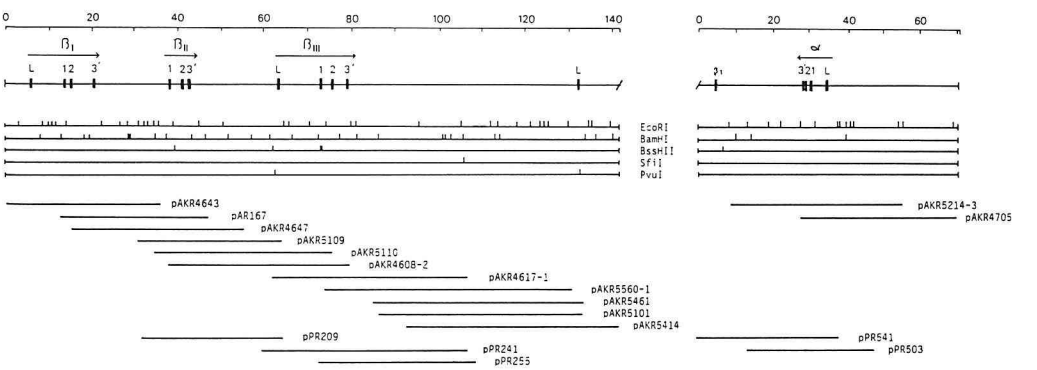

Fig. 8. (A) Molecular map of the DR subregion. The top line gives the scale in kb. The location of exons is shown by filled boxes. See the legend of Fig.4 for abbreviations. Overlapping cosmid clones were shown below the cleavage sites of the restriction enzymes.

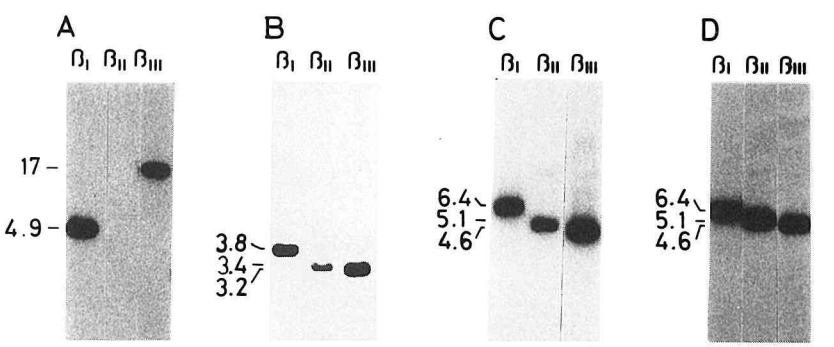

Fig. 9. Southern hybridization of the DRβ cosmid clones using domain-specific cDNA fragments as probes under non-stringent conditions. DNA from the cosmids pAKR4643(DRβI), pAKR4647 or pAKR5109(DRβII), and pAKR4617-1(DRβIII) were digested with EcoRI and electrophoresed. The probes used were pDRβ102 160bp PstI-SacI fragment(A), pDRβ101 (Inoko et al., 1985b) 140bp PstI fragment(B), pDRβ102 PstI 520bp fragment(C) and pDRβ102 290bp PstI fragment(D), which encode the leader, β_1, β_2+CY/TM and 3'UT region of the DRβ chain, respectively.

hybridized by the exon specific probes. The cross-hybridizations to other subregion genes was limited under the conditions used. Each probe gave two or three bands except for the result using the DRβ_1 domain specific probe. Some polymorphisms were observed between DR2Dw2(PGF) and DR2Dw12(AKIBA) using the leader sequence specific probe. The results are summarized in Table 1. No difference in the number of hybridizing bands between AKIBA and PGF, for example three bands using EcoRI and 3'UT probe, suggested that AKIBA and PGF have the same number of the DRβ gene exons. All hybridizing bands in AKIBA corresponded to the exon-containing region shown in the restriction maps of the DRβ region (Fig.8), and no additional band observed suggested no other DRβ gene exon than those cloned here. Then, I concluded that all of the DRβ genes was isolated.

The polymorphic DR antigen is encoded by the DRβIII gene

Two DRβ cDNA sequences were reported previously (Lee et al., 1987) and two DRβ chains were detected in two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) analysis (Nakatsuji et al., 1986; Nepom et al., 1984b; Sone et al., 1985). An acidic one is a polymorphic chain possibly encoding the Dw specificity, which has the variable amino acid sequences unique to the different Dw subtypes of DR2 (Dw2, Dw12 and Dw21) and the other basic one is a nonpolymorphic chain which has a more conserved amino acid sequence as well as isoelectric point. To distinguish the polymorphic from the nonpolymorphic gene, each cosmid containing the DRβI, βII or βIII gene was digested with a restriction endonuclease, BssHII, which recognizes the sequence "GCGCGC", and should cut in exon 2 (β_1 domain) of the polymorphic, not of the nonpolymorphic DRβ gene at the position of nucleotide 213 as predicted from the previously reported nucleotide sequence of the cDNA clones (Fig.11A), and subjected to Southern hybridization with the β_1 domain-specific probe(Fig.11B and C). Fig.11 showed that neither the DRβI nor the DRβII gene contained a BssHII site, but unexpectedly, the 3.2kb fragment encoding the β_1 domain of the DRβIII gene in pAKR4617-1 and pPR241 was cleaved to produce a 2.3kb fragment, establishing that the DRβIII gene had a BssHII site. Therefore, I can conclude

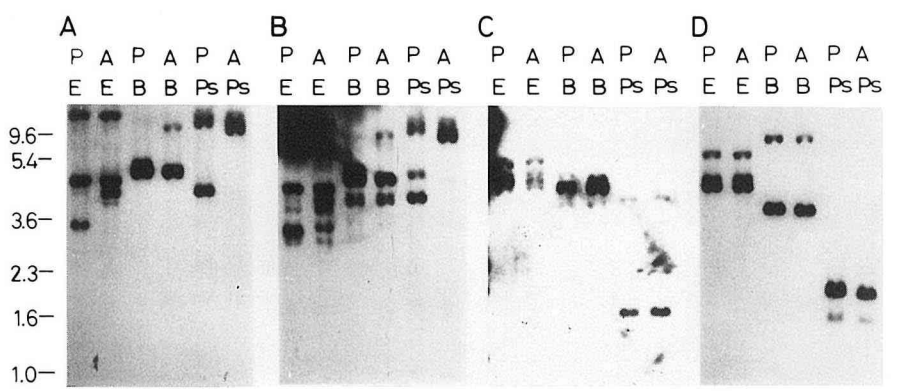

Fig. 10. Southern blot of genomic DNA using DRβ domain specific probes. High molecular weight DNA (7μg) from AKIBA and PGF were digested with the restriction endonucleases, EcoRI, BamHI and PstI, electrophoresed and transferred to nylon membranes. Probes used were the each domain specific fragment of pDRβ102 as follows: PstI-SacI 160bp fragment (leader sequence), PstI-RsaI 70bp (β_1 domain)(B), PstI 520bp fragment (the β_2 domain) (C) and PstI 290bp (3'UT) (D).

Table 1. Reactivity of the hybridizing bands with the DRβ-exon specific probes

probe[1)]	enzyme	size (kb)	PGF	AKIBA	expected gene-exon[2)]	estimated number of each exon
leader	EcoRI	17	+	+	βIII-L	
		4.9	+	+	βI-L	
		4.2		+	β'-L	
		3.0	+	+		3
	BamHI	14		+	β'-L	
		5.7	+			
		5.0	+	+	βI-L, βIII-L	
β_1	EcoRI	17	+	+	βIII-L	
		6.1	+	+	DRα-1	
		3.9		+	β'-L	
		3.8	+	+	βI-1	
		3.3	+	+	βII-1, βIII-1	4
	BamHI	14		+	β'-L	
		5.9	+		DRα-1	
		5.0	+	+	βII-1, βIII-L	
		4.2	+	+	βI-1, βIII-1	
β_2	EcoRI	6.4	+	+	βI-2	
		4.8	+	+	βII,βIII-2	
	BamHI	5.0	+	+	βII-2	3
		4.3	+	+	βI-2	
		3.9	+	+	βIII-2	
3'UT	EcoRI	6.4	+	+	βI-3'	
		5.1	+	+	βII-3'	
		4.6	+	+	βIII-3'	3
	BamHI	7.8	+	+	βI-3'	
		3.3	+	+	βII,βIII-3'	

[1)] Molecular size of the bands in kilobases.

[2)] Abbreviations for exons are as follows; L,leader sequence; 1,β_1 domain; 2,β_2 domain; 3',3'UT domain. DRα-1 expresses the isolated β_1 domain near the DRα gene. β'-L indicates the leader sequence down stream from the DRβIII gene.

that the polymorphic and nonpolymorphic DR2 antigens are specified by the DRβIII and DRβI genes, respectively, which is the reverse of the situation in other haplotypes such as DR3, DR4 and DR6 (Spies et al., 1985; Rollini et al., 1985; Gorski et al., 1985; Andersson et al., 1987; Tosi et al., 1986).

Arrangement of the three DRβ genes on chromosome 6

Inoko et al. (1988) constructed a large-scale genetic map of the HLA class II region in the DR2Dw12 haplotype (AKIBA) by pulsed field gel electrophoresis and found only two SfiI sites in the DR-DQ/DX subregions. One site was located between the DRα and β genes, and another between DXα and DVβ genes, giving the 330kb SfiI fragment shared by DVβ, DQβ, DQα, DRβI, DRβII and DRβIII genes. Therefore, I searched for an SfiI site in my DX-DVβ and DRβ cosmid clones, both SfiI sites, one was located 25kb downstream

from the DRβIII gene and another 6kb upstream from the DVβ gene were found (Fig.12). Thus, SfiI cleaved 10kb fragment of pA412 encoding DX–DV subregion and 6.2kb of pAKR5414 encoding downstream flanking region from DRβIII gene (Fig.12B). These results clearly indicated that the order of the class II genes in the DR2 haplotype was DXβ–DXα–DVβ–DQβ–DQα–DRβI–βII–βIII–DRα from the chromosomal centromere. This gene order and orientation of transcription in the DR subregion of the DR2 haplotype are identical to those of DR3 and DR4 as previously described (Spies et al., 1985; Rollini et al., 1985; Andersson et al., 1987).

Transfection and expression of DR genes in mouse L cells

I obtained two clones of the transfectants designated, L/DRα+βI and L/DRα+βIII, after separately cotransfecting pAKR4643(DRβI) and pPR241(DRβIII) together with pPR541(DRα), respectively. Analysis of the L/DRα+βI and L/DRα+βIII transfectants using flow microfluorimetry and a panel of anti-HLA class II specific MoAbs clearly showed successful transfection expression of the HLA-DR products on the cell surface. They reacted with the anti-DR MoAbs, L243 and Hu4 (Koide et al., 1982) (data not shown) but not with Leu10(anti-DQ) or B7/21(anti-DP) (Fig.13). Binding of the MoAb Hu30 to L/DRα+βI, but not to L/DRα+βIII is consistent with the result of 2D-PAGE studies showing that Hu30, which recognizes both DR1 and DR2 cells, is specific for the nonpolymorphic and supertypic DR2 antigen (Nishimura et al., 1984). Weak binding to the L/DRα+βI transfectant by the AB8.25 MoAb suggested that a common determinant recognized by the anti-DR2, DR5 and short DR3 MoAb, AB8.25, was shared by the nonpolymorphic DR2(DRα/DRβI), DR5 and short DR3 molecules. Neither of the two transfectants was found to react with the MoAb NDS15.38 which was previously described to be anti-DR2 (Makgoba et al., 1983).

Absorption of DR2 alloantisera by the DR gene-transfectants

To investigate which of the DRβI and DRβIII genes determines the so-called DR2 specificity for serological characterization of their products, the absorption assays was adopted using specific alloantisera. After absorption by the transfectants alloantisera were tested for cytotoxic ability against LCLs and B cell-enriched fractions from PBL as target cells. The alloantisera used in Fig.14, 1030, 1026 and 1029 were selected from the best sera against DR2 submitted to the 10th IHWS. Ex107, used as a negative control, is an anti-DR4 serum. As shown in Fig.14, L/DRα+βI and/or L/DRα+βIII significantly absorbed the DR2 alloantibodies of 1030, 1026 and 1029, but never of Ex107. L cells did not reduce the cytotoxic titer of these alloantisera. These results imply that the DR gene products expressed on the cell surface of the transfectants adopt a normal conformation. The alloantibodies, 1030 and 1026 were absorbed by both L/DRα+βI and L/DRα+βIII transfectants. On the other hand, 1029 was absorbed only by L/DRα+βI, suggesting that the DR2 determinants were comprised of at least two epitopes, and one was recognized by 1030 and 1026 residing on both the DR2(DRα/DRβI) and DR2(DRα/DRβIII) molecules, the other by 1029 only on the polymorphic DR2 molecule, the DRα/DRβIII heterodimer.

Four local alloantisera were also used in the transfectant absorption assay (Table 2). All of the DR2 alloantibodies tested were absorbed by both or either transfectants. Ex115 seemed to contain at least two alloantibodies, one against DR2 and the other against DR1 and/or DR4. A determinant recognized by Ex115 was located both on the nonpolymorphic

A

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a    GGG GAC ACC CGA CCA CGT TTC TTG CAG CAG GAT AAG TAT GAG TGT CAT   48
b    --- --- --- --- --- --- --- C-- TG- --- CC- --- A-G --- --- ---

a    TTC TTC AAC GGG ACG GAG CGG GTG CGG TTC CTG CAC AGA GGC ATC TAT   96
b    --- --- --T --- --- --- --- --- --- --- --- --- --- TA- T-- ---

a    AAC CAA GAG GAG AAC GTG CGC TTC GAC AGC GAC GTG GGG GAG TAC CGG  144
b    --- --G --- --- TC- --- --- --- --- --- --- --- --- --- T-- ---

a    GCG GTG ACG GAG CTG GGG CGG CCT GAC GCT GAG TAC TGG AAC AGC CAG  192
b    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

a    AAG GAC TTC CTG GAA GAC AGG CGC GCC GCG GTG GAC ACC TAC TGC AGA  240
b    --- --- A-- --- --G C-G GC- --G --- --- --- --- --- --- --- ---

a    CAC AAC TAC GGG GTT GGT GAG AGC TTT ACA GTG CAG CGG CGA          282
b    --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
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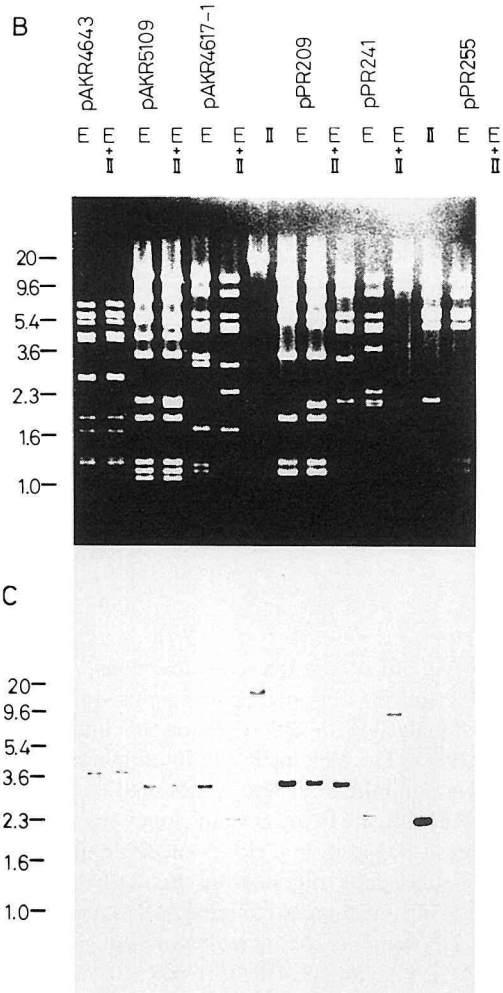

Fig. 11. (A) Nucleotide sequences of the β_1 domain of the two DRβ chains from the DR2Dw12 cell line (BGE) taken from published data(Lee et al., 1987). The polymorphic sequence is labeled "a", and the nonpolymorphic sequence is labeled "b". Dashes in the sequence "b" indicate identity with the sequences "a". The site cleaved(a) or not cleaved(b) by the restriction endonuclease, BssHII which recognizes the sequence "GCGCGC", is boxed. Electrophoresis (B) and Southern hybridization (C) of the DRβ genes singly digested by EcoRI(E) or BssHII(II), or double digested by EcoRI+BssHII (E+B) with the β_1 domain specific cDNA fragment as a probe. Each lane contained DNA from the cosmid clones pAKR4643(DRβI), pAKR5109(DRβII) and pAKR4617-1(DRβIII) derived from AKIBA, pPR209(DRβII) and pPR241(DRβIII) derived from PGF.

and polymorphic DR antigens. Ex99 could detect the common epitope expressed both on the DR1 and nonpolymorphic DR2(DRα/DRβI) molecule. JR21–224, like 1029, recognized the DR2 epitope specific for the polymorphic DR2(DRα/DRβIII) molecule. These results suggested that the DR antigens in DR2 had at least three allo-determinants defined by the alloantisera used here. One of them was the public or supertypic e.g., the common determinant both on the DR1 and DR2 antigens recognized by Ex99. The remaining two were the DR2 private determinants, one both on the DR2(DRα/DRβI) and DR2(DRα/DRβIII) heterodimers recognized by 1026, 1030 and Ex115, and the other one on the DRα/DRβIII heterodimer recognized by 1029 and JR21–224.

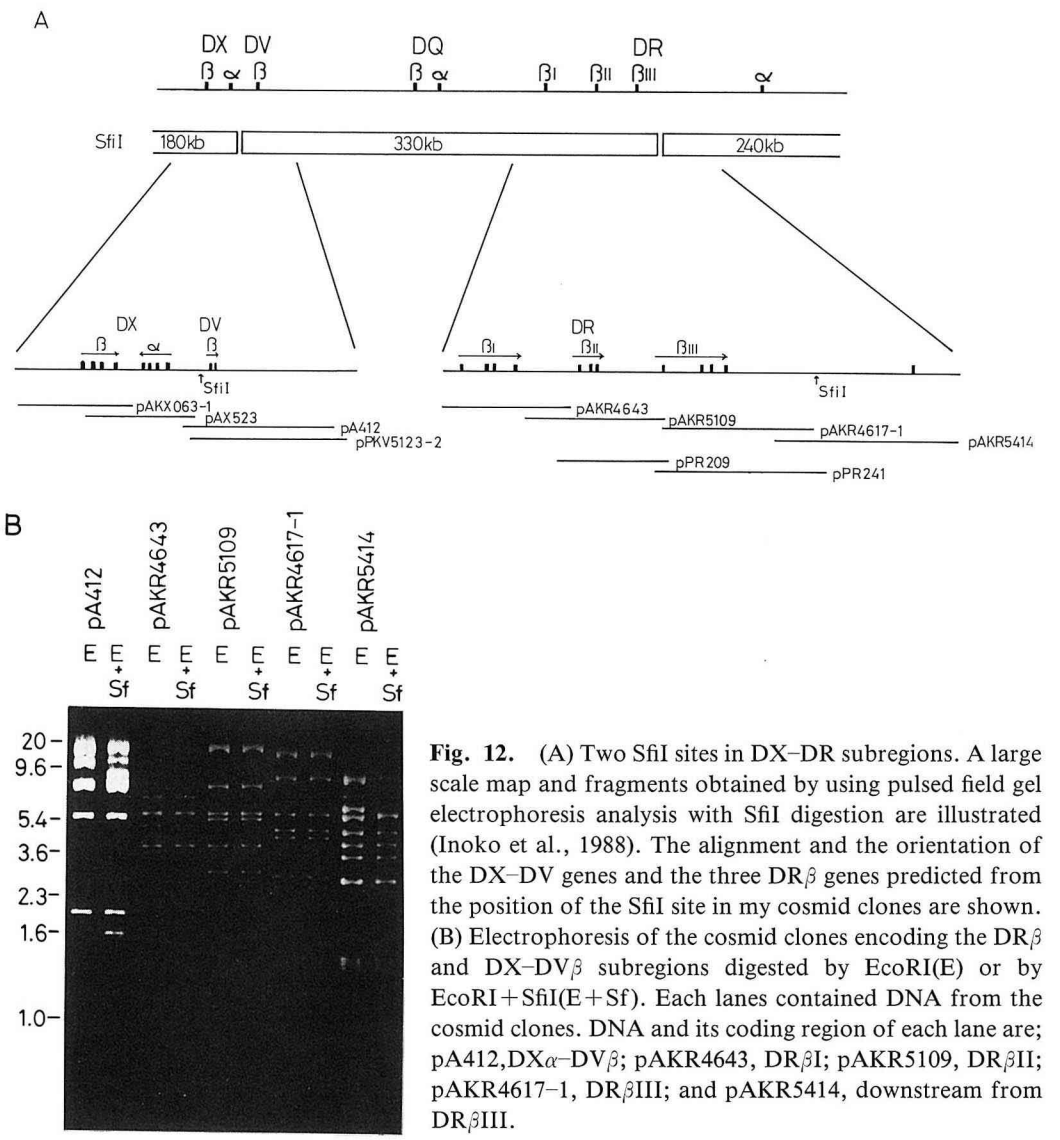

Fig. 12. (A) Two SfiI sites in DX–DR subregions. A large scale map and fragments obtained by using pulsed field gel electrophoresis analysis with SfiI digestion are illustrated (Inoko et al., 1988). The alignment and the orientation of the DX–DV genes and the three DRβ genes predicted from the position of the SfiI site in my cosmid clones are shown. (B) Electrophoresis of the cosmid clones encoding the DRβ and DX–DVβ subregions digested by EcoRI(E) or by EcoRI+SfiI(E+Sf). Each lanes contained DNA from the cosmid clones. DNA and its coding region of each lane are; pA412,DXα–DVβ; pAKR4643, DRβI; pAKR5109, DRβII; pAKR4617-1, DRβIII; and pAKR5414, downstream from DRβIII.

DISCUSSION

By screening the human genomic libraries constructed from HLA-homozygous B cell lines, AKIBA (DR2, Dw12, DQw1) and PGF (DR2, Dw2, DQw1), with the cDNA clones and successive gene-walking procedures, about 440kb DNA from the HLA-D region has been cloned and characterized.

Determination of encoding gene by isolated clones

Nucleotide sequences of HLA class II genes have conserved homologies, so that cross hybridization were observed both in the colony and in the Southern hybridization, Hybridization have been proceeded under the stringent condition to eliminate cross hybridization as possible. Moreover, short fragments of cDNAs, encoding the subregions which were low homology among whole HLA class II genes, were used as a probe, resulting successive isolation of genomic clones. Regions encoded by each isolated cosmid

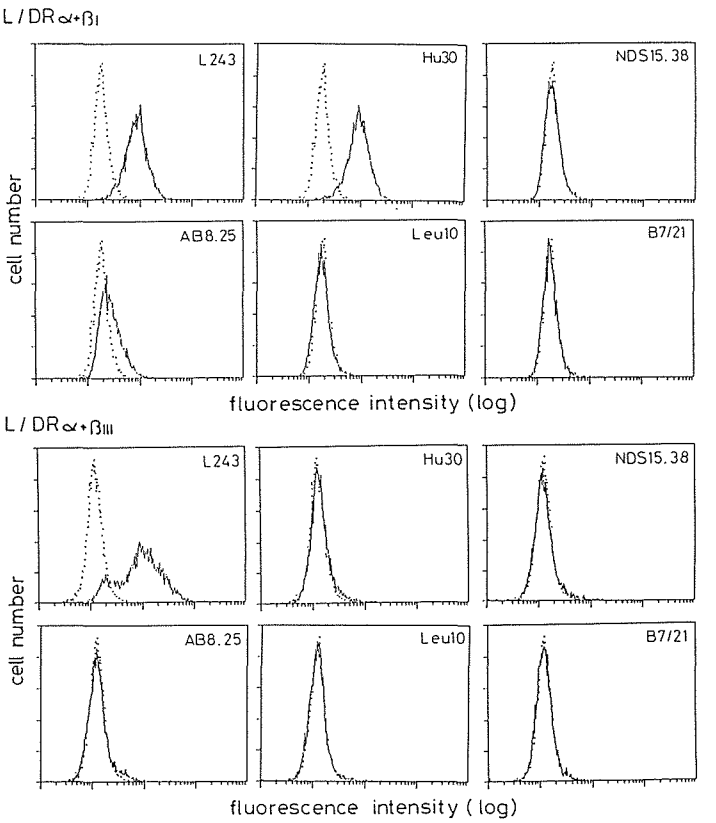

Fig. 13. Flow microfluorimetric analysis of the transfectant, L/DR$\alpha+\beta$I and L/DR$\alpha+\beta$III (which express the DRα/DRβI and DRα/DRβIII heterodimers on the cell surface, respectively) using a panel of anti-HLA class II MoAbs. The specificities of each MoAbs are: L243,HLA-DR; Hu30,HLA-DR1 and DR2; NDS15.38,HLA-DR2; AB8.25,HLA-DR2,DR5 and short DR3; Leu10,HLA-DQ and B7/21,HLA-DP. 5,000 cells were analyzed in each profile and plotted as log. fluorescence intensity against cell number by solid lines. In each profile, the negative control is the transfectant cell line stained with FITC rabbit anti-mouse Ig alone shown by dotted lines.

were determined using following strategies. (1) Homology between these cosmid clones and well-characterized cDNA clones using southern hybridization. (2) Comparison of restriction maps of isolated clones with previously published class II genes. (3) Some cosmid clones including class II genes were transfected into mouse L cells, and expressed products on their cell surfaces were detected by class II antigen-specific mAb.

HLA class II genes and their organization

I have cloned DOα, DXα,β, DVβ, DQα,β, and DRα,β genes from AKIBA and PGF. Exon-intron organization of DXα,β, and DQα,β genes are same to those of other haplotypes, (Okada et al., 1985; Jonsson et al., 1987), however, that of DR gene of DR2 haplotype has unique structure in comparison of other haplotypes.

DOα was reported to have less polymorphism. The restriction map around AKIBA DOα was identical to that of the DOα gene isolated from the genomic library of Maniatis (Inoko et al., 1985a). Localization of other class II gene α and βsuggest that a couple of α and β genes encoding class II antigen were found to be close each other, about 20kb. I could not, however, find β chain gene encoding β chain expected to associate with DOα

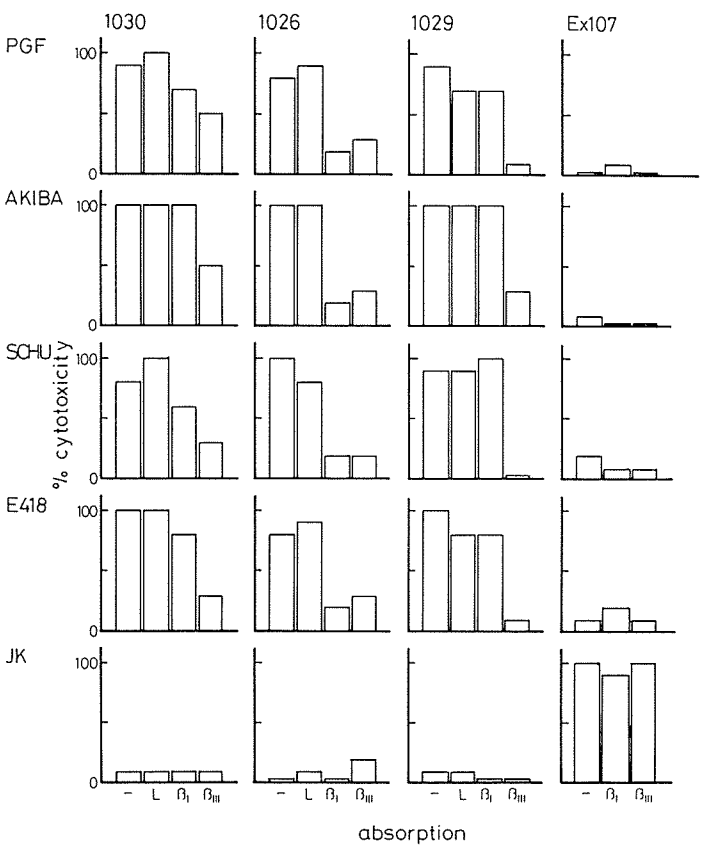

Fig. 14. Cytotoxic activities of various alloantisera after absorption by L cells(L), L/DRα+βI(βI) or L/DRα+βIII(βIII), or unabsorbed(-) against LCLs (PGF, AKIBA, SCHU and E418, DR2) and B cell-enriched fraction from PBL (JK, DR4/-) used as target cells. Specificities of alloantisera used are as follows, 1030, 1026 and 1029, DR2; Ex107, DR4.

Table 2. Microcytotoxicity analysis of anti-DR2 alloantisera obtained after absorption by each DR gene-transfectant cell.

Target cells[1]		%Cytotoxicity								
		Antisera[2]								
Designation	Phenotype	Ex115			Ex99			JR21-224		
		—[3]	βI[3]	βIII[3]	—	βI	βIII	—	βI	βIII
Sa	DR1	100	80	80	70	10	60	10	20	30
PGF	DR2	70	0	0	80	10	80	10	20	0
SCHU	DR2	90	10	10	100	20	100	80	90	20
AKIBA	DR2	80	20	30	100	30	80	90	90	20
E418	DR2	100	0	10	100	20	90	90	80	10
DEU	DR4	80	80	100	30	0	10	0	10	10
MANN	DR7	10	0	0		N.T.[4]			N.T.[4]	

[1] $3x10^3$ LCLs were used for cytotoxicity assay.
[2] Alloantisera specificities are as follows, Ex115, DR1,2,4,8; Ex99, DR1, 2short; JR21-224, DR2.
[3] Cells used for absorption, —, unabsorbed; βI,L/DRα+βI; βIII,L/DRα+βIII.
[4] Not tested.

chain. β chain gene associating with DOα is remained unclear.

DXα and β genes have high homology with DQα and β, respectively, at levels of nucleotide sequence, exon-intron gene organization, gene size and direction of transcription (Okada et al., 1985). No expression of DX gene, however, was reported until now. DXα and β genes have less polymorphisms among various haplotypes and Southern blotting pattern suggested no difference between the map of AKIBA (DR2,Dw12,DQw1) and of PGF (DR2,Dw2,DQw1). (Data not shown).

DVβ cosmid clones were obtained from the libraries of AKIBA and PGF (Fig.4A). Restriction map analysis and Southern blotting using locus specific probes revealed that DVβ gene located 15 kb upstream from the DXα gene. In addition to the string sequence homology of DVβ with DQβ (Ando et al., 1986a; and confirmed in Fig.4), mapping of the DVβ gene between the DX and DQ subregion strongly suggests that DVβ gene has been generated by gene duplication from the DXβ or DQβ gene or their common ancestral gene in the process of the class II gene evolution.

I cloned more than 90kb long region around DQα and β genes from two DR2DQw1 haplotypes, AKIBA (Dw12) and PGF (Dw2), and compared their gene organization, indicating the two characteristic differences. They are restriction site map polymorphisms around the DQβ gene and the deletion/insertion event upstream from the DQβ gene. Restriction site differences between these haplotypes which were concentrated near the DQβ gene, suggested the possibility of Dw typing by analysis of the DQβ gene region, and it was studied in accompanying paper (Kawai, 1990).

Dw specificities are determined by MLR, in which alloreactive T cells respond to proliferate against allogeneic class II antigens. Two reasons why DQ gene restriction map associates with Dw specificities. One is that Dw specificity are determined only by DR genes and restriction map polymorphisms are linked to the DR gene. The other is that DQ gene could present alloantigen to T cells. It was reported that HLA DQ antigen expressed on mouse L cells by gene transfection stimulate MLR responses (Nakatsuji, 1987) and DQ gene could control antigen specific immune response (Nishimura et al., 1990, Hirayama et

al., 1987).

I have cloned three DRβ genes by overlapping cosmid clones encompassing 140kb of continuous genomic DNA, and also one DRα gene from the DR2 genomic libraries (Fig.8). These clones include three DRβ genes, one DRα gene, and isolated two exons (one leader sequence and one β1 exon). Böhme et al. (1985) suggested that three DRβ genes existed in the DR2 haplotype. Indeed, digestion with EcoRI, BamHI and PstI followed by hybridization with the 3'UT specific probe revealed the presence of three bands in genomic DNAs isolated from AKIBA(DR2,Dw12) and PGF(DR2,Dw2), corresponding to each of the three DRβ genes cloned here (Fig.10 and Table II), suggesting that these cosmid clones spanned the entire DRβ subregion.

The organization of the HLA class II genes in the DR2 haplotype was determined to be centromere–DX–DVβ–DQ–DRβI–βII–βIII–DRα–telomere (Fig.12). This arrangement and the orientation of three DRβ genes is similar to other haplotypes such as DR3 and DR4(Spies et al., 1985; Rollini et al., 1985; Andersson et al., 1987). The similarity extends to the restriction maps around the DRα gene, the existence of the isolated β_1 exon next to the DRα gene, the SfiI site downstream from the DRβIII gene (Inoko et al., 1988) and of the isolated DRβ leader sequence downstream of the DRβIII gene (Fig.8). This conservation of arrangement suggests that the establishment of the organization of the region preceded the evolutionary diversification of the DR haplotypes. On the other hand, the differences in the gene structure around the DRβIII gene, such as the lack of the DRβ leader sequence in DR2 which was identified 20kb downstream from the DRβIII gene in DR3 and DR4, and the absence of the leader sequence in the DRβII gene, must reflect a degree of independent DRβ gene organization in some of the DR haplotypes (Fig.15).

Antigenicity of the two DR antigens

Restriction enzyme digestion and transfection experiments clearly showed that the DRβI and DRβIII genes encode the nonpolymorphic and polymorphic DRβ chain, respectively. This DRβI gene corresponds to the nonpolymorphic gene product which reacts with the supertypic MoAb, Hu30 after association with the DRα chain. On the other hand, mRNA from the DRβIII gene corresponds to the polymorphic cDNA containing the BssHII site in the β_1 exon, and its product does not react with Hu30 (Fig.11 and Fig.13). Although in other haplotypes such as DR4, DR3 and DR6 (Spies et al., 1985; Rollini et al., 1985; Gorski et al., 1985), the nonpolymorphic or supertypic DRβ chain, which encoding DRw52 and DR253 specificity, was demonstrated to be encoded by one of the most telomeric DRβ genes (DRβIII in DR3,6, and DRβIV gene in DR4), in DR2 the DRβI gene represents the nonpolymorphic DRβ chain (Fig.15). These facts indicate that the susceptibility to mutations or gene conversions responsible for genetic polymorphism or diversity does not depend on the location of gene on the chromosome, but is a property of the gene itself. Furthermore this factor controlling the susceptibility might exist either within the gene or near it, because it can affect about 10kb area surrounding it.

How has the apparent inversion of DRβ genes happened? Two mechanisms are possible. 1) reciprocal intergenic exchange between the DRβI and βIII genes. Reciprocal genetic exchange might have frequently occurred between the two DRβ genes, resulting in generation of a polymorphic DR2 allele in the DRβIII gene, together with creation of the nonpolymorphic DRβI gene. Wu et al. (1986) suggested reciprocal intergenic exchange responsible for generation of Dw21 (Dw"FJO") from Dw12. 2) change of the susceptibility

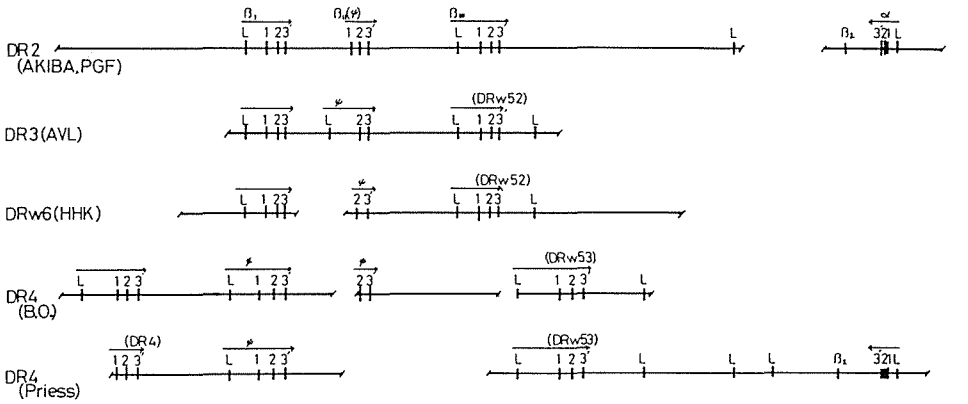

Fig. 15. Comparison of the DR gene region structure among various haplotypes. References for other haplotype than DR2 as follows; DR3(AVL), Rollini et al. (1985); DRw6(HHK), Gorski et al. (1987); DR4(B.O.), Andersson et al. (1987); and DR4(Priess), Spies et al. (1985). See the legend of Fig.4 for abbreviations.

to gene exchange, divergence or mutation. After establishment of the DR gene structure by a succession of gene duplications, divergence and gene conversion, one gene might have changed to be variable and other gene to be conservative. In either case, these events might undergo concurrent diversification of the DR2 haplotypes, because both AKIBA and PGF with the same DR2 but with different Dw specificities (Dw12 and Dw2, respectively) similarly had the polymorphic DRβIII gene and the nonpolymorphic DRβI gene.

I described here that the single DR antigen in DR2 had multiple determinants and the so-called DR2 specificities were determined by several epitopes, some of which were expressed on both the polymorphic and nonpolymorphic DR antigens (Fig.14 and Table II). Similarly, in other haplotypes, it has been established that some DR antigenic determinants exist on two DR molecules distantly-related to each other. For example, the nonpolymorphic DRw52 and polymorphic DR3 molecules share the common determinant recognized by the MoAb, Tr81 (Berte et al., 1988). Although Maeda and Hirata (1984) reported that the DR2 alloantiserum precipitated two DR molecules in 2D-PAGE analysis, the possibility remains that the alloserum contained polyclonal antibodies. Indeed, anti-DR1, 3, 4 and 8 antiserum, Ex115, was showed to containing polyclonal antibodies, in which anti-DR2 antibodies reacted both with polymorphic and nonpolymorphic DR2 antigens (Table II). Here, we could clearly show that both of the two DR molecules had the same allo-determinant as evidenced by the absorption assays with the transfectants expressing the single DR molecule. The fact that all DR2 sera selected here except Ex99 antiserum recognized at least the polymorphic DR molecule implies that the DR2 haplotype can be mainly defined by the allo-determinants residing on the polymorphic DRβ chain. An epitope recognized by Ex99 resides on both DR1 and nonpolymorphic DR2 antigens, indicating supertypic antigen feature correspond to DRw52 and DRw53 of other haplotypes.

Serum absorption assay suggesting the multiple determinants on the two DR molecules indicate that there are possibly three types of restriction by the HLA-DR antigens in immune responses, first restriction to the polymorphic DR molecule, second to

the nonpolymorphic DR molecule, and third to both of two molecules. Wilkinson et al. (1988) clarified that *M.leprae*-specific T cell clones isolated from leprosy patients could use only the polymorphic DRβ chain as their restriction molecule but not the nonpolymorphic DRβ chain by using the DR-transfected mouse L cells.

General discussion

In this study, I analyzed gene structure and antigenicity of the HLA class II antigens in DR2. Large number of genomic clones including most of class II genes, DR, DQ, DX, DV and DO genes, were isolated. Previously, DP region have been isolated by Ando et al. (1986b) from DR2. Although DRβ genes vary in number between different specificities, pulse field gel electrophoresis analysis suggested that the organization of different HLA haplotypes is apparently very similar, thus, DP, DO, DX/DV, DQ and DR (Weiss and Ziegler, 1989; Hardy, 1986). Length of clones isolated from DR2 until now, encompasses through 500kb, which is about half of whole class II region. There is possibility that some gene(s) other than the HLA genes exist in these cloned region and that it affect the development of non-autoimmune, HLA-associated disease. Genes of the cytokines tumor necrosis factors α and β (Dunham et al. 1988; Carroll et al., 1987), the heat shock protein HSP70 (Sargent et al., 1989), and two HLA-B associated transcript genes (Banerji et al., 1990) were described in the HLA region. Gene insertion-deletion event observed in the DQβ gene upstream region might associate structure and expression of 'disease susceptibility gene'. On the other hand, in the study of HLA-associated autoimmune disease, mouse L cell transfectants expressing either DQ or DR antigen will be available. Furthermore, since antigen presenting cells, such as dendritic cells and macrophages, express all of HLA class II antigens, DR, DQ and DP on their cell surfaces, it is hard to dissociate and analyze each function of those class II antigens. It will be possible to understand the molecular basis for the immune response and MHC-disease association by use of the DR-and DQ-transfected cells described here.

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