A Physical Map Including a New Class I Gene (cda12) of the Human Major Histocompatibility Complex (A2/B13 Haplotype) Derived from a Monosomy 6 Mutant Cell Line

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To avoid interpretative problems due to restriction fragment length polymorphisms, the monosomy 6 mutant cell line BM19.7 was employed to establish a molecular map of the human major histocompatibility (HLA) complex in the A2,B2,Dw4,DRw6,DRw52,-DQw1,DPw2 haplotype. Results were obtained mainly by field-inversion gel electrophoresis and Southern blotting techniques. The map extends to 4800 kb and includes the HLA complex with a length of 4200 kb. Five HTF islands could be positioned on the map. The class I region has a size of about 2000 kb and includes nonclassical HLA class I genes, some of which must be localized within 200 kb telomeric of HLA-A. A new class I gene, cda12, distinct from HLA-A, HLA-B, or HLA-C, has been localized within 50 kb from HLA-A. The class I region contains a gap of about 500 kb, just telomeric of HLA-C, in which further class I genes could not be detected. The class II region has a size of 1000 kb, which is separated from the class I region by about 1200 kb. The 5' end of the HLA-B gene is situated centromeric, giving an orientation opposite to that of the TNFA and TNFB loci. The estimated length of the HLA complex correlates well with its size determined cytogenetically using mutant cell lines with interstitial deletions.

INTRODUCTION

The human major histocompatibility (HLA) complex is located on the short arm of chromosome 6 in the region 6p21.31-6p21.33 (Spring et al., 1985). There are three clusters of genes, the HLA class I, II, and III regions. The class I and II genes encode cell surface glycoproteins and show an extreme degree of polymorphism. The class I loci consist of at least 17 highly related genes (Strachan, 1987), including those encoding the classical transplantation antigens HLA-A, -B, and -C. At least 15 genes are included in the class II region and code for α and β chains of the respective antigens (Trowsdale, 1987). The class III region contains a cluster of genes specifying the complement components C2 and C4 and factor B as well as two copies of the steroid 21-hydroxylase gene (Carroll et al., 1984, 1985). In addition, the loci for tumor necrosis factor (TNFA) and lymphotoxin (TNFB) map between the class III and the class I regions as determined by pulsed-field or field-inversion gel electrophoresis (PFGE and FIGE, respectively) (Dunham et al., 1987; Ragoussis et al., 1988).

Precise knowledge of the arrangement of HLA genes and the physical distances involved is essential for an understanding of several important properties of the HLA system, e.g., the association of haplotypes with a variety of diseases and the phenomenon of linkage disequilibrium between alleles of different loci (Bodmer, 1987). Physical maps of the HLA complex have so far been presented for only two different haplotypes, namely, A2,B2,D1 (Lawrance et al., 1987) and A2,B7,DR2 (Dunham et al., 1987; Carroll et al., 1987), mainly by using HLA homozygous cell lines and concentrating on the class II/class III region. A physical map of the class II region of a DR4,Dw4 cell line has also been presented (Hardy et al., 1986). We have employed monosomy 6 cell lines (Ziegler et al., 1985a,b) to obtain a molecular map of the HLA complex as well (Ragoussis et al., 1986; Ziegler et al., 1988). So far, however, the available physical mapping data of the class I region are tentative. This is due to the few locus-specific DNA probes and the lack of overlapping restriction enzyme-generated DNA fragments found in PFGE. Therefore, we focused on the region from the class III loci to the telomeric part of the class I gene region, employing the monosomy 6 mutant BM19.7.
with an A2,B13,Bw4,C-,DRw6,DRw52,DQw1,DPw2 haplotype.

To measure the physical length of the HLA complex, we assigned genes to fragments as long as 1500 kb, making use of recent improvements in the methods for separation of large DNA molecules (Smith et al., 1987; Vollrath and Davies, 1987). Furthermore, we were able to localize a new HLA class I gene in the vicinity of the HLA A locus.

**MATERIALS AND METHODS**

**Cell Lines**

The following cell lines were used: BJAB-B95.8.6 lymphoma cell line (HLA haplotypes A1, Cw4, B35, Bw6, DR5, DRw52, DQw3, DPw4 and A2, C-, B13, Bw4, DRw6, DRw52, DQw1, DPw2) and the mutant cell line BM19.7. This line was established by selecting HLA-Bw6-locus variants from γ-irradiated BJAB-B95.8.6 cells with a monoclonal antibody and complement. Cytogenetic, isozyme, and Southern blotting analyses of the cloned subline BM19.7 demonstrate that it retains only the A2-bearing chromosome 6 (Ziegler et al., 1985a,b).

**FIGE AND "ROTAPHOR" Analysis of Genomic DNA**

Low-melting-point agarose blocks containing cells from cell line BM19.7 or BJAB-B95.8.6 (10⁶ cells/block) were prepared according to Van der Bliek and co-workers (1986) by a procedure modified slightly for FIGE (Ragoussis et al., 1988; Carle et al., 1986). FIGE was carried out for 48-60 h at 100 V and 15°C. The voltage gradient was inverted periodically with a time ratio of 3 to 1 and with the longer time increasing from 10 to 180 s. A computer-controlled power supply (CS 130, version 2.1) was employed (EMBL, Heidelberg, FRG).

In addition to FIGE, separation of DNA molecules with sizes larger than 1000 kb was performed in an apparatus (“ROTAPHOR,” Biometra, Göttingen, FRG) as described (Ziegler et al., 1987). A 20-cm² gel (1% agarose) was placed in a 33-cm² apparatus with rotating electrodes. An electronically controlled step motor moved the electrodes around the gel, with an angle of 120° between the orientations of the electric field. The interval (pulse time in other designs) was varied between 60 and 2200 s, the field strength was 2-5 V/cm, and the running time was 43-144 h at 14°C, depending on the size of the DNA fragments to be separated. After a run, the gel was stained in ethidium bromide (1 µg/ml) for 30 min, destained in H₂O for 30 min, and photographed under 302-nm uv light.

As molecular size markers, *Saccharomyces cerevisiae* strains WAY5-4A and DBY 747, *Schizosaccharomyces pombe*, and λ concatemers were prepared as described (Vollrath and Davies, 1987; Schwartz and Cantor, 1984; Van Ommen and Verkerk, 1986). We estimate that the size determinations of the large DNA fragments are correct within ±10%; estimates obtained using different techniques (FIGE, ROTAPHOR, CHEF (Vollrath and Davies, 1987)) gave similar results.

The DNA was blotted (Southern, 1975) to Amersham Hybond membranes (Amersham-Buchler, Braunschweig), baked for 2 h at 80°C, and hybridized sequentially with various DNA probes. Prehybridization, hybridization with [³²P]dCTP-labeled DNA probes (Feinberg and Vogelstein, 1983), and washing were performed as described by Ragoussis et al. (1988).

**Probes**

As an HLA-B locus-specific probe, a 5.0-kb *KpnI/HindIII* fragment (pCD2.6KH5) located 29 kb 5’ of the HLA-B gene was isolated (Weiss et al., 1988). Another B-locus-specific probe (pCD2.6T2.2), isolated 26 kb 3’ of HLA-B, was employed as well. The probe pCD2.6PE2.25, a 2.25-kb *EcoRI/PstI* HLA-B gene fragment used as an HLA-B/C cross-hybridizing probe, is located just 3’ of HLA-B (Weiss et al., 1988). pMF28, a full-length 1.5-kb HLA-B cDNA, was employed as an HLA class I cross-hybridizing probe, and pEX1.35, a 1.35-kb *XbaI/EcoRI* fragment flanking the 3’ end of HLA-A2, as an HLA-A-specific probe. Two probes were used to map the novel HLA class I gene cda12: a single-copy probe (1.2-kb 5’ flanking *KpnI/XhoI* fragment) and a low-copy probe (1.5-kb intragenic fragment). These probes were isolated from cosmids cda12 (Fig. 1), which leads to cell surface expression of a novel HLA class I heavy chain after transfection into murine L929 and P815 cells (Messer et al., manuscript in preparation). The cda12 cosmid was obtained by screening the cd library constructed from DNA of the haplotype HLA-A2,-B27/B51,-Cw2/Cw3 with the HLA-A locus-specific probe (Weiss et al., 1985).

Probes for C2 and C4 (class III region) were kindly provided by Dr. D. R. Campbell and Dr. P. Schneider (Belt et al., 1984; Campbell and Bentley, 1985). The TNFA probe pCAH5SH2.9 was a HindIII/SalI fragment derived 29 kb 5’ of the TNFA gene (Kioussis et al., 1987; Ragoussis et al., 1988). Another probe (pCAH5KB5) flanking the 5’ end of the TNFR locus

![Restriction map of the cda12 cosmid with the origin of the two probes (open bars) used for mapping the cda12 gene (arrow). H, HindIII; X, XhoI; K, KpnI; C, ClaI; S, SalI.](image-url)
and two probes (pCAH5P6 and pCAH5P11) from the 3' region of the TNFA locus were also used.

The origin of the class II probes has been described (Ragoussis et al., 1986).

RESULTS

Organization of the Class II Region

Our results indicate an overall length and organization of this haplotype identical to those already published (Dunham et al., 1987; Carroll et al., 1987; Lawrance et al., 1987; Hardy et al., 1986; Ragoussis et al., 1986). It is striking that the very long NotI fragment from the DX genes to C2 (920–1000 kb according to various authors), the NotI fragment extending from DP to DOB, and a 1200-kb NruI fragment covering the whole class II region have been consistently found in different haplotypes: DR2 (Dunham et al., 1987; Carroll et al., 1987), DR1 (Lawrance et al., 1987), DR4 (Hardy et al., 1986), and DRw6 (this paper) (Fig. 2).

Organization of the Class III Region

Including TNFA/B

The only restriction enzyme (RE) fragment so far found hybridizing to the 3'-TNF locus probes pCAH5P6 and pCAH5P11 as well as to the class III region probes (C2 and C4) is a 650-kb NruI fragment (Figs. 3a and 3b). The 5'-TNF genomic probes pCAH5SH2.9 and pCAH5KB5 hybridized to a 50-kb NruI fragment (Fig. 3c). All probes derived from the 5' and 3' regions of TNFA and TNFB detected the same 1100-kb NotI, 950-kb SalI, 370-kb BssHII (Figs. 3b and 3c), 100-kb MluI, and 150-kb SfiI fragments (not shown), which did not include C2 or C4. Double digests revealed a 270-kb NotI/NruI and a 300-kb SalI/NruI fragment hybridizing to TNF 3' and 5' region probes and not to C2 or C4 (Figs. 3a, 3b, and 3c and not shown), indicating a 300-kb minimal distance between C2 and TNFA (Fig. 2). The 1100-kb NotI and the 950-kb SalI and 370-kb BssHII fragments all hybridized to a probe derived 29 kb from the 5' end of HLA-B (pCD2.6KH5) (Fig. 3d) and to all TNF/A/B locus probes (Figs. 3b and 3c). Probes derived from the HLA-D gene or 25 kb from its 3' end hybridized to a 250-kb BssHII fragment (not shown, identical to “270 kb” in Ragoussis et al., 1988). Both BssHII fragments were included in the 1100-kb NotI fragment. Double digests with BssHII and NruI gave a 250-kb fragment hybridizing to the 5' HLA-B probe (Fig. 3d) and a 20-kb next to a 50-kb fragment hybridizing to the 3'-TNF probes (Fig. 3b), thus indicating that the BssHII fragment exceeds the
FIG. 3. FIGE separation of DNA from the monosomy 6 mutant cell line BM19.7 digested with the RE NotI (N), NruI (R), SalI (S), and BssHII (B). "X" indicates double digestion with the respective enzymes. FIGE conditions were 100 V, 10–180 s time ramp, 3:1 ratio, 60 h at 15°C. The Southern blot was hybridized sequentially with C2, C4 (a), the 3'-end TNFA probes (b), the 5'-end TNFB probes (c), the 5'-end HLA-B locus probe (d), the HLA-A-specific probe (e), and the class I cross-hybridizing probe (f). Molecular size markers: λ concatemers and yeast chromosomes.

TNF loci by 70 kb toward the class III region and that the distance between TNFB and HLA-B is about 250 kb.

Organization of the Class I Region

The 1100-kb NotI, 950-kb SalI, and 250-kb BssHII fragments and a 550-kb NruI fragment hybridized to pCD2.6PE2.25, a probe cross-hybridizing to HLA-C (not shown). MluI gave a 150- and a 100-kb fragment (present also in Fig. 4a), the latter hybridizing also to B-locus-specific probes. The 150-kb MluI fragment was included in the 250-kb BssHII fragment (not shown). Since the HLA-B gene is localized very close to the centromeric end of the BssHII fragment, the minimal distance between HLA-B and HLA-C is 100 kb and the maximal distance 250 kb. This value is in excellent agreement with data presented by Pontarotti et al. (1987) and Chimini et al. (1987).

A full-length class I cDNA probe (pMF28) cross-hybridizing to all class I genes, including RS-5 (Sri-
vastava et al., 1987; E. Weiss, unpublished results), was used to detect RE fragments in addition to those already known to include HLA-B or HLA-C. The following new fragments were found: a 1400- and a 150-kb NotI fragment; a 1300-kb NruI fragment; MluI fragments of 1500 as well as 800 kb; three Sall fragments with lengths of 350, 250, and 50 kb, respectively; and a 420-kb BssHII fragment (Fig. 3f and Fig. 4a). From these new fragments, the 1500-kb MluI, 1400-kb NotI, 1300-kb NruI, 420-kb BssHII, and 350-kb Sall segments included the HLA-A gene, since an HLA-A locus-specific probe detected only those (Fig. 3e and Fig. 4b). The length of the very long DNA fragments was determined by using the ROTAPHOR. The 150-kb NotI, 800-kb MluI, as well as the 250- and 50-kb Sall fragments must contain class I sequences other than HLA-A, -B, and -C. A single NruI fragment (1300 kb) contained all detectable class I genes except the HLA-B/C region, linking HLA-A to “nonclassical” HLA class I genes.

The probes for the functional HLA class I gene cda12 (Messer et al., manuscript in preparation) hybridized to the same NotI, NruI, and MluI fragments as the HLA-A specific probe. HLA-A and cda12 were located also on the same 50-kb Sall fragment and 250-kb BssHII X Sall double-digestion product (Figs. 5a and 5b). Therefore, the cda12 gene must be situated within 50 kb in the vicinity of the HLA-A locus.

The arrangement of class I gene-containing RE fragments becomes evident after an analysis of double- and triple-digestion products. A NruI X NotI double digestion and hybridization to pMF28 give a 150-kb fragment which also appears when NotI is employed alone (see above and Fig. 4a). However, in the triple-digest NotI X NruI X MluI (Fig. 4a), the 800-kb MluI fragment
fragment known to carry only nonclassical class I genes is also reduced in length, to 150–180 kb (Fig. 4a). This indicates that at least one nonclassical class I locus, in addition to cdal2, must be positioned at one end of the 1300-kb NruI fragment, which overlaps with the 800-kb MluI and includes the 150-kb NotI fragment.

The approximate position of the HLA-A locus in the NotI, NruI, and MluI fragments was determined in the same way (Figs. 3e, 4b, 6a, 6b): NotI X NruI digestion gave a 1100-kb HLA-A harboring fragment, while NruI X MluI resulted in a 1000-kb piece. Triple digestion using these enzymes did not reduce the length of the 1000-kb fragment any further (Figs. 4b and 6a), showing that the single-digestion products overlap extensively (Fig. 2). In some cases, a further NotI site was recognized in the 1400-kb fragment, giving an approximate 600-kb product, containing HLA-A. In that case, further digestion with MluI resulted in a 500-kb fragment (Fig. 6b). Thus the 1500-kb MluI product containing HLA-A must be localized 300 kb from that end of the 1300-kb Nru1 fragment, which contains the nonclassical class I loci (Fig. 2). Double digests with NruI, MluI, NotI, BssHII, and Sall show that the HLA-A gene is located within 300 kb of the telomeric part of the 1500-kb MluI fragment (Figs. 3e, 3f, and 6a). In combination with data presented by others (Dunham et al., 1987; Carroll et al., 1987), the most likely arrangement of this cluster of fragments is presented in Fig. 2. Assuming that there are no additional large MluI fragments in that region, the distance between HLA-C and HLA-A is about 1200 kb. In the unlikely case that the orientation of the entire class I gene cluster is opposite to that shown in Fig. 2, the HLA-C to HLA-A distance would still be 900 kb.

DISCUSSION

"Nonclassical" Class I Genes

The functional HLA class I gene cdal2 is distinct from all class I heavy-chain sequences (Messer et al., unpublished results) known so far, including the HLA-E gene. While the distance between HLA-E and HLA-C is about 650 kb (Carroll et al., 1987), the cdal2 gene is located very close to HLA-A (within 50 kb). The cdal2 gene will be useful as an additional marker for recombination studies in the class I region. We suggest the designation "HLA-F" for this locus.

Additional nonclassical class I genes must be situated 150–200 kb telomeric of HLA-A. There seem to be no further class I genes present centromeric to HLA-B. Thus, all the nonclassical class I genes map telomeric of HLA-B, and most likely also telomeric to HLA-C.

Physical Map and Disease Associations

Our results demonstrate that the size of the entire HLA complex is about 4200 kb, while the individual regions have lengths of 1000 kb (class II), 1200 kb (to HLA-B), and 2000 kb (HLA-B to telomeric end of the class I region). The distance between HLA-B and HLA-A has been shown to be about 1300 kb in the A2,B7 (Carroll et al., 1987) and A2,B27 (Lawrance et al., 1987) haplotypes. We estimate about 1400 kb for the A2,B13 haplotype, a region long enough to include many more genes, some of which may be associated with diseases (see also Tiwari and Terasaki, 1985, for review). It would be particularly interesting to analyze the HLA haplotypes in which alleles of more than two loci are in strong linkage disequilibrium, so-called "complotypes" (Awdeh et al., 1983).

The distances between HLA loci determined by recombination in families (Lamm and Olaisen, 1985) are generally in agreement with the physical mapping data. However, discrepancies are apparent for three regions: The HLA-C-A, DR-DQ/DX, and DQ/DX-DP genetic distances differ significantly depending on the method by which they were determined, assuming that generally 1 cM equals 1000 kb. Suppression of recombination and recombination hot spots may be responsible for these phenomena.

A comparison of the RE fragments detected by different groups, even in similar haplotypes, shows that only a few RE fragments are always identical. The most striking case is the 950-kb NotI fragment covering the region from DOB to C2. The differing size of RE fragments will complicate the analysis of HLA haplotypes
from diseased individuals, as the regions of interest will have to be mapped de novo.

**HTF Islands in the HLA Complex**

A comparison of the RE sites present in the DNA of all cells analyzed so far allows the localization of HTF islands (Bird, 1986; Lindsay and Bird, 1987), which are thought to be associated with transcriptionally active regions of chromatin. There are at least five such regions within the HLA complex: between DX and DOB close to DOB, between DRA and C4, between C2 and TNFα close to C2 (Dunham et al., 1987), within 50 kb telomeric to HLA-C, and within 200 kb telomeric to HLA-A (Fig. 2).

**Correlation of Physical Mapping and Deletion Mapping Data**

Our estimate of the size of the HLA complex is in very good agreement with data derived from cell lines with interstitial deletions affecting HLA genes (Spring et al., 1985; Erlich et al., 1983; Gladstone et al., 1982). By comparison of mutants with various overlapping deletions, the HLA complex must represent 2–3% of the entire length of chromosome 6. Since chromosome 6 has a length of about 165,000 kb (derived from 3 × 10^6 bp/haploid genome and the more than average size of chromosome 6), a value of 4125 kb represents 2.5% of its size. Obviously, this value correlates very well with the FIGE RE-mapping data.

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