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Mapping of the genes encoding the HLA-DR α chain and the HLA-related antigens to a chromosome 6 deletion by using genomic blotting

(cDNA hybridization probes/major histocompatability complex/restriction fragment polymorphism)

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ABSTRACT **We have used genomic blotting with DNA from a human cell line that has a small deletion on chromosome 6 (6.3.6) and from its parent cell line (T5-1) to map DNA fragments complementary to cloned DNA sequences encoding the HLA-B7 antigen (class I) and the α chain of the HLA-DR antigen (class II). The 6.3.6 variant fails to express the *HLA-A*, *-B*, *-C*, and *-DR* and *MB* specificities associated with one of the parental T5-1 haplotypes and has a visible deletion in the short arm of one chromosome 6 (1). The gene locus assignment was based on the expectation that, if the chromosomal location of the DNA sequences used as a hybridization probe were within the deletion, then the relative amount or size (or both) of genomic restriction fragments that hybridize to the probe in T5-1 and in 6.3.6 DNAs should differ predictably. By comparing the genomic blot patterns from T5-1 and 6.3.6 DNAs, we have shown directly that the loss of haplotype expression was due to deletion of the structural genes and have mapped the structural gene for the HLA-DR α chain to the chromosomal location (6p2105–6p23) defined by the 6.3.6 deletion. A cDNA clone encoding the α chain of the HLA-DR antigen hybridized to two genomic fragments, 4.2 and 3.8 kilobases long, generated by *Bgl* II digestion of T5-1 DNA. The 4.2-kilobase fragment was absent from DNA derived from the 6.3.6 deletion variant. Thus, this fragment could be assigned to the parental chromosome 6 with the *A1*, *B8*, *DR3* haplotype, and the 3.8-kilobase fragment, to the chromosome 6 with the *A2*, *B27*, *DR1* haplotype. In addition, comparison of the T5-1 and 6.3.6 genomic blot patterns obtained with the *HLA-B7* probe revealed dosage differences for all of the class I genomic fragments generated by *Bam*HI digestion, suggesting that all of the class I loci map to the region 6p2105–6p23.**

The major histocompatibility complex (MHC) consists of a set of tightly linked genes encoding cell surface glycoproteins that are involved in various immunological functions (1–3). The human MHC, the HLA complex, is located on the short arm of chromosome 6 and contains two distinct multigene families. The class I genes code for the classical transplantation antigens, HLA-A, -B, and -C, which are composed of a polymorphic 45,000-dalton glycoprotein associated with a nonglycosylated 12,000-dalton invariant chain, β_2-microglobulin, not encoded by the MHC. These antigens are present on the surface of all nucleated cells. In addition, a number of related glycoproteins which are associated with β_2-microglobulin (TL, Qa) but show a restricted tissue distribution have been described in mouse cells (4–6) and, more recently, in human cells (7, 8). The class II genes (the immune response genes) code for the Ia antigens, heterodimers composed of a 34,000-dalton (α) and a 29,000-dalton (β) glycopeptide present primarily on B lymphocytes and macrophages (9–13). These serologically defined proteins, the human Ia or the HLA-DR antigens, map to the *HLA-D* region, initially defined by the mixed lymphocyte reaction (11–13). The results of two-dimensional electrophoresis suggest that the serologic polymorphism resides in the HLA-DR β chain (11, 13); no electrophoretic polymorphism for the HLA-DR α chain has been detected and, consequently, it has proved difficult to map the structural gene definitively. The order of genetic loci in the MHC is centromere, glyoxylase I, *D/DR*, *B*, *C*, and *A*.

The advent of recombinant DNA technology has made possible the physical isolation of eukaryotic genes. Recently, genomic and cDNA clones encoding the human MHC class I and II antigens have been identified and characterized (14–20). These clones can be used as probes to localize the cognate structural genes. By using antibody selection techniques, somatic cell variants that fail to express the HLA specificities associated with one of the parental haplotypes have been isolated and characterized (21, 22). The availability of those variants which result from deletions, in conjunction with cloned MHC DNA sequences, allows for the precise mapping of MHC structural genes to the chromosomal location defined by the deletion. The MHC hemizygous variant, 6.3.6, differs from the parent T5-1 by containing a small terminal deletion on the short arm of one of the chromosome 6 homologs.

We have used the technique of genomic blotting, with several different restriction endonucleases, to map genes to the 6.3.6 deletion. This analysis is based on the expectation that, if the chromosomal location of the DNA sequences used as a hybridization probe were within the deletion, then the relative amounts or the size (or both) of specific genomic restriction fragments derived from T5-1 and 6.3.6 DNAs should differ predictably. This approach represents a general method for mapping cloned DNA sequences by using individual chromosomal deletions and genomic blotting.

MATERIALS AND METHODS

Cell Culture. T5-1 is a clonal subline derived from B-LCL PGLC33H (23). It has the haplotypes *HLA-A1*, *-B8*, *-DR3*, and *HLA-A2*, *-CW1*, *-B27*, *-DR1* (1) and is heterozygous for phosphoglucomutase 3 and homozygous for glyoxylase I-1 (GLOI). The 6.3.6 deletion variant, which expresses only the haplotype *HLA-A2*, *-CW1*, *-B27*, *-DR1*, was isolated and characterized as described (1). Both cell lines were grown in RPMI 1640 medium plus 15% fetal bovine serum and 20 mM Hepes buffer.

Genomic Blotting. High molecular weight genomic DNA was prepared by using a modification of described procedures (24).

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Abbreviations: kb, kilobase(s); NaCl/Cit, 0.15 M NaCl/0.015 M sodium citrate, pH 7.0; MHC, major histocompatibility complex; GLOI, glyoxylase I-1.

Restriction endonuclease (New England BioLabs) digests of genomic DNA were precipitated with ethanol and electrophoresed through a 0.6% agarose gel at 30 V for 18 hr. DNA in the gel was partially depurinated prior to transfer in 75 mM HCl for 15 min at 20°C, permitting complete transfer within 3 hr (25). Transfer of DNA to nitrocellulose was carried out by the method of Southern (26). The nitrocellulose filters were hybridized with the nick-translated probe (27) as described (28) and washed at 20°C in 2× standard saline citrate (NaCl/Cit; 1× is 0.15 M NaCl/0.015 M sodium citrate, pH 7.0)/0.1% $NaDodSO_4$ and subsequently at 50°C in 0.1× NaCl/Cit containing 0.1% $NaDodSO_4$ and analyzed by autoradiography.

For quantitative analysis, specific amounts of genomic DNA, measured by absorbance at 260 nm, were digested and electrophoresed. The relative amounts of DNA in the gel were monitored by ethidium bromide staining before and after transfer to nitrocellulose. The relative amount of genomic fragments transferred to the filter was monitored by hybridizing the blot with a probe known to map outside the deletion and quantitating the autoradiographic band intensity by densitometry. The probe used was p4E1, a cDNA clone encoding β1 interferon (David Mark, personal communication) which maps to chromosome 9 (29).

RESULTS AND DISCUSSION

Origin and Cytology of the Somatic Cell Variants. The 6.3.6 variant and the T5-1 subline from which it was derived have been described in detail (21). The T5-1 parent line is heterozygous for chromosome 6 markers (*HLA-A1, -B8, -DR3/-A2, -CW1, -B27, -DR1*) and has a terminal deletion of the short arm of chromosome 6, with the breakpoint at the band 6p23. The 6.3.6 line has a further deletion of the short arm of the shorter chromosome 6 and is *HLA* hemizygous (*HLA-A2, -CW1, -B27, -DR1*) but has the parental (T5-1) level of glyoxylase activity, a marker (*Glo1*) between the centromere and the *HLA* complex (Fig. 1). The breakpoint of the 6.3.6 deletion is between 6p2105 and 6p22 and lies between the *Glo1* and *HLA-DR* loci (1).

Mapping Strategy. The strategy for mapping specific DNA sequences to the 6.3.6 deletion is based on the assumption that, if the pattern of specific genomic restriction fragments differs between DNAs derived from the T5-1 and 6.3.6 lines, then the observed difference in genomic blot patterns can be attributed to the chromosome 6 deletion in the 6.3.6 variant. If the specific genomic fragments resulting from restriction endonuclease digestion are polymorphic, then the T5-1 DNA will contain two fragments of different lengths, detected as autoradiographic bands, each fragment being derived from an individual chromosome homolog (Fig. 2*A*). If a specific probe hybridizes to sequences within the deletion, then the 6.3.6 DNA will contain one band derived from the nondeleted chromosome 6 and will lack the band derived from the chromosome 6 bearing the deletion. The band in the 6.3.6 lane will have the same intensity as the corresponding band in the T5-1 lane.

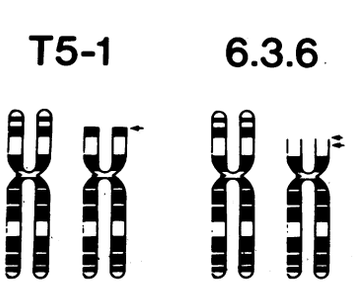

FIG. 1. Diagram of G-banded karyotypes of chromosome 6 of T5-1 and 6.3.6 (21). Single arrow indicates breakpoint of 6p23 in T5-1; double arrow indicates breakpoint region (6p2105–6p22) in 6.3.6.

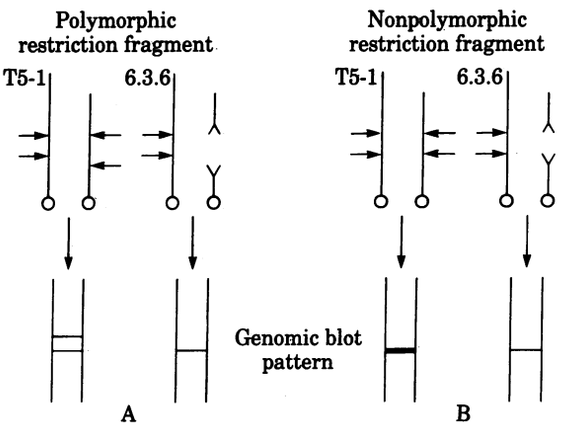

FIG. 2. Diagram of predicted genomic blot pattern for DNA sequences located within the deletion on the short arm of chromosome 6. Horizontal arrows represent restriction endonuclease cleavage sites.

If, on the other hand, the genomic restriction fragments are not polymorphic, then the T5-1 DNA will contain a band derived from both chromosome 6 homologs whereas the 6.3.6 DNA will contain a band of reduced intensity derived only from the nondeleted chromosome 6 (Fig. 2*B*). Thus, the absence or the reduced intensity of a 6.3.6 band relative to the corresponding T5-1 band maps the genomic fragment to the 6.3.6 deletion. The quantitative analysis of genomic blot band intensities requires appropriate control experiments (described below) to ensure that, in fact, equal amounts of genomic DNA are being compared.

A genomic blot pattern in which a specific band derived from 6.3.6 DNA is absent from T5-1 DNA is also theoretically possible. A band unique to 6.3.6 DNA would be expected if the deletion breakpoint were located within DNA sequences hybridizing to the probe, generating a new "junction" fragment. No such bands unique to 6.3.6 DNA were observed in this study.

Class II (*HLA-DR α*) Genes. Genomic blots of *Bgl* II-digested DNA from T5-1 and 6.3.6 were hybridized with a nick-translated cDNA probe encoding the HLA-DR α chain (Fig. 3*A*). The pDRα-1 cDNA clone, which was isolated with a synthetic oligonucleotide probe and identified by DNA sequence analysis, encodes the entire α chain of the HLA-DR antigen (19). The T5-1 DNA contains *Bgl* II fragments of 4.2 and 3.8 kilobases (kb) which hybridize to the probe; the 6.3.6 DNA contains only the 3.8-kb fragment. Because other restriction endonucleases generate fragments of identical length from both chromosome 6 homologs in T5-1 (ref. 19; unpublished data), the variation in the *Bgl* II fragment length can be attributed to restriction site polymorphism rather than to insertions or deletions. The *Bgl* II digestion pattern indicates that the cell line T5-1 is heterozygous for a polymorphic *Bgl* II site close to or just within the *HLA-DR α* gene and, furthermore, that the 4.2-kb fragment is derived from the chromosome 6 with the *A1, B8, DR3* haplotype whereas the 3.8-kb fragment is derived from the chromosome 6 with the *A2, B27, DR1* haplotype. In agreement with this conclusion is our recent finding that a different T5-1 variant with a lesion in the *HLA-D* region of the opposite *A2, B27, DR1* haplotype has lost the 3.8-kb fragment (unpublished data). The observed genomic blot pattern involving the disappearance of a polymorphic restriction fragment formally maps the *HLA-DR α* gene to the chromosomal location defined by the 6.3.6 deletion. This result is consistent with the assignment of the *HLA-DR α* gene to chromosome 6 on the basis of ge-

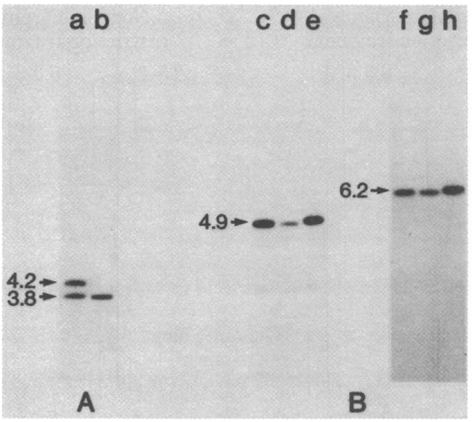

FIG. 3. Restriction endonuclease-digested DNA from 6.3.6 and T5-1 hybridized with ^{32}P-labeled *HLA-DR* α (class II) and interferon-β1 probes. (*A*) DNA (7.0 μg) from T5-1 (lane a) and 6.3.6 (lane b) cells was digested with *Bgl* II, fractionated, blotted, hybridized with the nick-translated ^{32}P-labeled *HLA-DR* α probe, and autoradiographed. (*B*) Eight micrograms of DNA from T5-1 (lanes c and f) and 6.3.6 (lanes d and g) and 16.0 μg of DNA from 6.3.6 (lanes e and h) cells were digested with *Xba* I and fractionated, blotted, and hybridized sequentially with the *HLA-DR* α probe (lanes c, d, and e) and with the interferon-β1 probe (lanes f, g, and h). Arrows denote fragment size (in kb). The faint lower bands resulted from a previous hybridization of the same nitrocellulose filter with a class I probe and represent residual probe incompletely removed prior to hybridization with the *HLA-DR* α clone.

nomic blotting with DNA from somatic cell hybrids (ref. 17; unpublished data) and demonstrates that the *DR* α locus is tightly linked to the *HLA* region.

Most restriction endonuclease cleavage sites in or near the *HLA-DR* α gene are nonpolymorphic (19). However, quantitative comparison of the T5-1 and 6.3.6 genomic blot patterns can be used to localize genes even when the probe hybridizes to a single restriction fragment of identical size in T5-1 and 6.3.6 DNA (Fig. 3*B*). For quantitative comparison of probe hybridization to specific genomic fragments, equal amounts of DNA from T5-1 (lanes c and f) and 6.3.6 (lanes d and g), and twice the amount of 6.3.6 DNA (lanes e and h) were digested with *Xba* I, fractionated by electrophoresis, and transferred to nitrocellulose. The nitrocellulose blot was hybridized sequentially with a nick-translated class I probe, the *HLA-DR* α probe, and a control probe known to map outside the deletion (Fig. 3*B*).

To monitor the relative amounts of genomic DNA transferred to the nitrocellulose filter and available for hybridization, we used an interferon-β1 cDNA clone, p4E1 (D. Mark, personal communication) as a control hybridization probe. The interferon-β1 gene has been mapped to chromosome 9 (29) and therefore the relative autoradiographic intensities resulting from hybridization of the p4E1 probe to specific genomic fragments in lanes f, g, and h of Fig. 3 reflect the amount of genomic DNA bound to the filter. The interferon-β1 band intensities, quantitated by densitometry, were used to normalize the relative intensities of the *HLA-DR* α bands (see Table 1).

A single *Xba* I fragment, 4.8 kb long, hybridized to the *HLA-DR* α probe in both T5-1 (lane c of Fig. 3) and 6.3.6 (lanes d and e). The intensity of the T5-1 band (lane c) is about twice that of the 6.3.6 band (lane d) but is approximately equal to the band that resulted from probing twice the amount of 6.3.6 DNA (lane e). These autoradiographic results, quantitated by densitometry (Table 1), are in good agreement with the expected ratios and are consistent with the assignment of the *HLA-DR* α gene to the 6.3.6 deletion, inferred from the *Bgl* II digestion pattern. Because the HLA-DR α chain shows limited polymorphism, it has previously been difficult to map the gene based on analysis of the protein product.

Table 1. Relative amount of probe hybridization

Probe	DNA source		
	T5-1	6.3.6	6.3.6, double concentration
HLA-DR α	2.0	0.7	1.9
Interferon-β1 cDNA	2.0	1.4	4.0
HLA-DR α normalized	1.0	0.5	0.48

The relative autoradiographic intensities of the T5-1 and 6.3.6 bands in Fig. 3*B* were quantitated by densitometry. To normalize for the amount of DNA bound to the nitrocellulose filter, the relative intensities obtained with the *HLA-DR* α probe for a given lane were divided by the relative intensities obtained with the interferon-β1 probe.

The genomic blot data obtained with the *HLA-DR* α probe under stringent hybridization conditions (Fig. 3) (17, 19) suggest that the HLA-DR α chain is encoded by a single-copy locus but cannot exclude the possibility of duplicated loci with conserved restriction sites. Because the two *Bgl* II fragments of *HLA-DR* α from T5-1 are derived from different chromosomes, any hypothesis invoking multiple loci with conserved restriction sites would require the additional assumption that the *Bgl* II sites on one chromosome are conserved but are different from the conserved *Bgl* II sites on the other chromosome. Thus, the observed *Bgl* II genomic blot pattern for T5-1 and 6.3.6 is difficult to reconcile with any hypothesis other than a single-locus model.

Hybridization of the cDNA bank from which the *HLA-DR* α clone was derived (19) with the *HLA-DR* α probe under conditions of reduced stringency revealed a number of cDNA clones distinct from the *HLA-DR* α clone. These clones represent candidates for related sequences encoding additional α chains. We have tested one such clone as a probe for T5-1 and 6.3.6 DNA; it hybridizes to sequences that are distinct from the *HLA-DR* α gene but are located within the region defined by the 6.3.6 deletion (unpublished data). The identification of cDNA clones encoding additional α chains is consistent with recent serological and biochemical studies implicating at least two distinct *HLA-D*-region-encoded, heterodimeric cell surface antigens (30, 31). We have also mapped genomic sequences hybridizing to *HLA-DR* β cDNA clones as well as to a cDNA clone encoding the HLA-DR γ chain, a polypeptide associated with HLA-DR immunoprecipitates, which appears to be the human equivalent of the mouse I_i polypeptide (32, 33). The HLA-DR β sequences map within and the *HLA-DR* γ sequences map outside the deletion (unpublished data). This approach represents a general method for examining the genetic linkage of loci encoding the polypeptide chains of a complex antigen.

Class I (*HLA-A, -B, -C*) Genes. We have used the cDNA clone encoding the HLA-B7 antigen (15) to probe *Bam*HI, *Pvu* II, and *Xba* I digests of genomic DNA from the T5-1 and 6.3.6 cell lines (Fig. 4). Digestion with each restriction endonuclease generated multiple fragments which hybridized to the probe, a result consistent with the reported DNA sequence complexity of the class I multigene family (34, 35). In the *Bam*HI digest (lanes a, b, and c), the probe hybridized to genomic fragments of identical size in DNA from T5-1 and 6.3.6, indicating that the distribution of *Bam*HI sites in the class I genes is the same (nonpolymorphic) on both chromosome 6 homologs. However, the autoradiographic intensity of each band is reduced in 6.3.6 DNA (lane b) relative to T5-1 DNA (lane a), when equal amounts of genomic DNA were probed. Bands of equal intensity are seen when twice the amount of 6.3.6 DNA (lane c) is compared with T5-1 DNA. These are the expected results if the *HLA* hap-

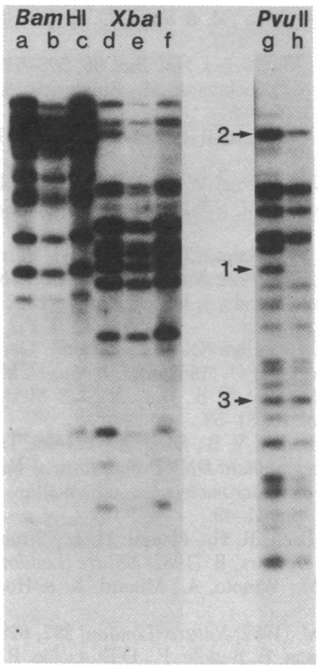

FIG. 4. Restriction endonuclease-digested DNA from 6.3.6 and T5-1 hybridized with ^{32}P-labeled *HLA B7* (class I) probe. DNA—8.0 μg from T5-1 cells (lanes a, d, and g) and from 6.3.6 cells (lanes b, e, and h) and 16.0 μg from 6.3.6 cells (lanes c and f)—was digested with *Bam*HI, *Xba* I, and *Pvu* II, fractionated, blotted, and hybridized with the *HLA-B7* probe. The arrows indicate bands in the *Pvu* II digest which are unique to T5-1 DNA (arrow 1), of reduced intensity in 6.3.6 DNA (arrow 2) and of equal intensity in T5-1 and 6.3.6 DNAs (arrow 3).

lotype loss in the 6.3.6 variant is due to hemizygosity rather than to homozygosity generated by mitotic crossing over.

The gene dosage differences for class I and class II loci observed here confirms the analysis of the 6.3.6 variant based on cytology and antigen dosage (21). Moreover, because every band shows a reduced intensity in 6.3.6 DNA, the *Bam*HI digestion pattern suggests that all of the class I genomic fragments hybridizing to the *HLA-B7* probe are derived from the region deleted in the 6.3.6 variant. This result is consistent with the finding that, in family studies, all of the polymorphic class I bands segregate with unique parental *HLA* haplotypes (unpublished data). In addition, this result is of considerable interest because the majority of the genomic fragments hybridizing to the class I probes do not encode the classic transplantation antigens, HLA-A, -B, and -C, and their murine analogs (35, 36). Many of the class I genomic fragments encode the murine Qa and TL antigens and their human equivalents; some of the class I genomic sequences have been shown to be "pseudogenes"—i.e., loci which, for various structural reasons, are incapable of encoding an intact gene product (37). In some multigene families, like the globins, pseudogenes are located on a different chromosome from the rest of the globin family members (38). The genomic blot pattern observed here, however, indicates that all members of the class I multigene family are clustered and map within the region deleted in the 6.3.6 variant.

In the *Pvu* II and the *Xba* I digests, polymorphic restriction fragments were revealed by genomic blotting, indicating that the distribution of restriction sites on the two chromosome 6 homologs is different. In the comparison of T5-1 and 6.3.6 class I genomic fragments, three banding patterns are seen. For a given T5-1 band, the corresponding 6.3.6 band is either absent, of reduced intensity, or of equal intensity. The absence of a 6.3.6 band results from a restriction fragment polymorphism and indicates that the T5-1 band is derived from a region on the shorter chromosome 6 that was deleted when the 6.3.6 variant was derived; this result formally maps the T5-I band to the deletion. The reduced intensity of the 6.3.6 band indicates that both chromosome 6 homologs contain the same genomic fragment but that 6.3.6 DNA contains only half as much of this specific fragment as T5-1 DNA; this result also formally maps the genomic fragment to the deletion. Bands of equal intensity can result either from fragments that map outside the deletion (two copies in both T5-1 and 6.3.6) or from a unique polymorphic fragment derived from the nondeleted chromosome 6 (one copy in both T5-1 and 6.3.6) that maps to the deletion. The latter interpretation of the equal intensity of the *Pvu* II and *Xba* I bands is more likely because all of the *Bam*HI bands map to the deletion. Moreover, in both the *Xba* I and *Pvu* II digests the length of DNA (in kb) contained within the equal-intensity bands is roughly equivalent to the length of DNA in the polymorphic bands unique to T5-1 DNA. This relationship might be expected if the equal-intensity bands were derived from the deletion (Fig. 2*A*).

The apparent linkage of all the class I loci may reflect either recent gene duplications or selective pressure to maintain linkage. In gene families with identical members (e.g., ribosomal RNA), the maintenance of homogeneity within the individual loci has been attributed in part to gene conversion. In families with homologous but distinct members, like the *HLA* class I sequences, gene conversion has been postulated to account for the pattern of genetic polymorphism (37, 39, 40) and, in addition, also might be expected to prevent individual loci from diverging too far from the canonical sequence. If the multiplicity of class I loci represents a genetic reservoir for polymorphism maintained by gene conversion (37), selective constraints may prevent dispersion.

In summary, all of the multiple class I genomic restriction fragments as well as the gene for one of the class II antigens (*DR-α*) appear to map between bands 6p2105 and 6p23 by the genomic blotting method described here. This approach represents a general method for mapping cloned DNA sequences to chromosomal sites defined by deletion variants. In addition to the chromosomal localization of genes encoding characterized products, this method is capable of identifying new genes encoding previously unidentified products that map to the deletion. The analysis of cDNA or genomic clones by using the 6.3.6 deletion provides an opportunity for identifying new MHC-linked genes. We have identified two different cDNA clones from our B cell-specific cDNA bank (19) that encode proteins distinct from the class I and class II antigens which hybridize to genomic sequences within the 6.3.6 deletion (unpublished data).

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