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# Genetic mapping of a human class II antigen β-chain cDNA clone to the *SB* region of the HLA complex

(molecular cloning/major histocompatibility complex/polymorphism/HLA-D)

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**ABSTRACT** **A class II antigen β-chain cDNA clone was isolated from a human B-cell cDNA library by using as a probe the murine *I-Aβ* gene. This cDNA clone, pHAβ, was shown to be distinct from the *DCβ*- and *DRβ*-related loci by DNA sequence analysis, thus suggesting that it might correspond to a third polymorphic human class II locus, *SB*, which encodes secondary B-cell antigens. Genetic mapping of this β-chain cDNA clone to the *SB* region was performed by the blot hybridization procedure. We showed that (*i*) within panels of HLA-DR homozygous human B-cell lines and of unrelated individuals who have been typed for HLA antigens, differential mobility of DNA fragments segregated with distinct *SB* genotypes; (*ii*) γ-ray-induced deletion mutants that have lost the expression of DR or DC/MT antigens but maintain SB expression preserved a pattern consistent with (*a*) their SB phenotype and (*b*) the genetic independence of the *SB* locus with respect to DR and DC/MT; and (*iii*) within an informative family, two siblings differing only for one allele at the *SB* locus (because of the occurrence of an internal recombination between DR and GLO) and otherwise HLA identical exhibited a restriction enzyme polymorphism linked to the *SB* locus. Therefore, all available data are compatible with identity between *HAβ* and *SBβ*.**

In man, the highly polymorphic *HLA-D* region of the major histocompatibility complex (MHC), whose products have been primarily detected on B lymphocytes and macrophages, controls the major part of the proliferative response in mixed lymphocyte reaction (MLR). Serological as well as cellular reagents allowed the identification of two sets of molecular entities that display similar function and tissue distribution to HLA-D products. These antigens, named DR and DC, have been separated and are each composed of two noncovalently associated subunits, a heavy (α) and a light (β) chain. DR and DC are the structural equivalents of murine I-E and I-A antigens, respectively. Among the other serologically defined specificities is the MT series found in strong linkage disequilibrium with HLA-DR. Its molecular relationship to the DR and DC antigens is not well defined, but MT1 has been shown to be identical to DC1 (for review, see refs. 1 and 2).

Lymphocytes from individuals that type identically for all known HLA antigens, including DR and DC/MT, have been found to be capable of reciprocal MLR, suggesting the existence of at least one additional MLR locus. The study of recombinant families in which positive MLR was detected between cells of genotypically *DR,DC/MT*-identical individuals allowed the division of the *D* region, between *DR* and *GLO*, into two subregions. These subregions were separated by recombination into a telomeric region coding for the DR and DC/MT antigens and a centromeric region coding for a new segregant series called SB (3–7). Further analysis of informative recombinant families (8) as well as γ-ray-induced deletion mutants that have lost DR and DC/MT expression but maintained the SB expression (9, 10) clearly established the genetic and functional independence of the SB gene products from DR and DC/MT and mapped the *SB* locus centromeric to *DR,DC/MT* and telomeric to *GLO* loci, respectively. To date, six alleles of SB antigens have been defined (6, 7) and population studies (11–13) pointed out no significant linkage disequilibrium between SB and DR specificities.

In this report, we describe the molecular cloning of a human class II β chain, HAβ, previously called DYβ (14), distinct from DRβ and DCβ as assessed by nucleotide sequence analysis. Its genetic mapping to the *SB* region has been accomplished by (*i*) population studies, (*ii*) studies of γ-ray-induced deletion mutants that have lost the expression of DR or DC/MT antigens but maintain SB expression (9, 10), and (*iii*) studies of an informative recombinant family (3).

## MATERIALS AND METHODS

**Cloning and Sequence Determination.** The library of cDNA clones constructed from the human lymphoblastoid B-cell line JY (DR4,w6) has been described (15). Screening was performed in nonstringent conditions (15) with a radioactive probe derived from the murine *I-Aβ* gene (16). One of the faintly hybridizing clones was further characterized by mapping with various restriction endonucleases (New England BioLabs). Nucleotide sequence was determined by the procedure of Maxam and Gilbert (17), either with end-labeled restriction fragments or after subcloning restriction fragments in pUC9 or pUC12 (18) and end-labeling the cleaved polylinker (18).

**Cell Lines.** Typing of D/DR homozygous cell lines (19) for SB antigens was kindly performed by S. Shaw (National Cancer Institute). Human peripheral blood lymphocytes (PBL) were typed for HLA-D antigens (Table 1) by using HLA-DR workshop antisera and SB NIH standard primed lymphocyte typing reagents (11). HLA antigen-loss mutants were generated by γ-ray irradiation of the human lymphoblastoid B-cell line LCL721 (A2,1; B5,8; DR1,3; MT1,2; SB2,4) as described (10, 20).

**Southern Blots.** High molecular weight DNA was prepared as described (21). DNA digested by restriction endonuclease was analyzed by electrophoresis on agarose gel and transferred to nitrocellulose filters (Schleicher & Schuell) according to the

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Abbreviations: MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; PBL, peripheral blood lymphocytes; kb, kilobase(s); bp, base pair(s).

method of Southern (22). After transfer, the filters were baked, prehybridized, and hybridized with a radioactive probe labeled with $^{32}P$ by nick-translation (23). The hybridization was performed at 65°C for 12–16 hr as described (19). Low stringency conditions: after incubation, the filters were washed four times in 0.75 M sodium chloride/0.075 M sodium citrate, pH 7/0.2% $NaDodSO_4$ for a total time of 2 hr and then washed two times in 0.15 M sodium chloride/0.015 M sodium citrate/0.2% $NaDodSO_4$ for 1 hr. High stringency conditions: in addition, the filters were washed two times in 15 mM sodium chloride/1.5 mM sodium citrate/0.2% $NaDodSO_4$ for 1 hr. All washes were carried out at 65°C. The filters were then exposed by using Kodak XAR-5 film and DuPont Lightning Plus intensifying screens or Quanta III Plus double screens.

## RESULTS AND DISCUSSION

**Identification and Sequence Determination of a cDNA Clone Encoding a Human Class II Antigen β Chain.** A 2.1-kilobase (kb) *Bam*HI DNA fragment corresponding to the 5′ end of the *I-Aβ* gene (16) was used to screen a library of cloned cDNA derived from mRNA enriched for the class II antigen mRNAs of the human JY cell line (15). Of the 4,500 colonies screened, 6 were found to hybridize with the probe. One of these clones, pHAβ, was subjected to complete sequence analysis by the method of Maxam and Gilbert (17). The nucleotide and deduced amino acid sequences are presented in Fig. 1. The cDNA insert of pHAβ is 780 base pairs (bp) long, and the first 545 bp encode the portion of a class II antigen β chain from residue 51 through the carboxyl terminus. This was determined by matching the first three cysteine residues of HAβ with cysteines 79, 117, and 172 of an HLA-DRβ chain (26) and introducing a single gap at position 150 in the DRβ chain sequence. The termination codon (TAA) is followed by a 235-bp 3′-untranslated region [excluding the poly(A) tract].

**Amino Acid Sequence Comparison of HAβ Chain With the DRβ and the DCβ Chains: Identification of a Third Molecular Species.** Recently the complete sequence of cDNA clones encoding class II antigen β chains has been reported (24, 25). Amino-terminal (27) and complete (26) amino acid sequence data on isolated class II antigens made it possible to distinguish between cDNA clones that encode DCβ (24) and DRβ (25). To evaluate the relatedness of the protein coded for by pHAβ and the other class II antigen β chains, the sequence of HAβ was aligned with the amino acid sequences of DRβ and DCβ (Fig. 1). Assuming no length differences in the extracellular portion, these comparisons involve 75% of the extracellular sequences. HAβ differed from both DCβ and DRβ in 55 and 64 of 181 positions. The overall homology between HAβ and DCβ and DRβ was 71% and 65%, respectively. Likewise, comparisons between the DRβ and the DCβ sequences, from residues 51 to the carboxyl terminus, displayed an overall homology of 68%. Therefore, HAβ appears to correspond to a third isotypic form of class II antigen β chain.

Class II antigen β chains are composed of four domains: two extracellular domains, each containing a disulfide loop, a membrane spanning region, and a cytoplasmic domain. The sequences of partial amino-terminal (residues 51–95) and complete carboxyl-terminal (residues 96–189) extracellular domains in all three molecules were compared. The homology found between DCβ or DRβ and HAβ first extracellular domains (β1) was 69% or 62%, respectively. All three chains differed at eight positions, of which five differences were found clustered in a segment spanning positions 84–89. For the remaining differences two of the chains were alike while one was different.

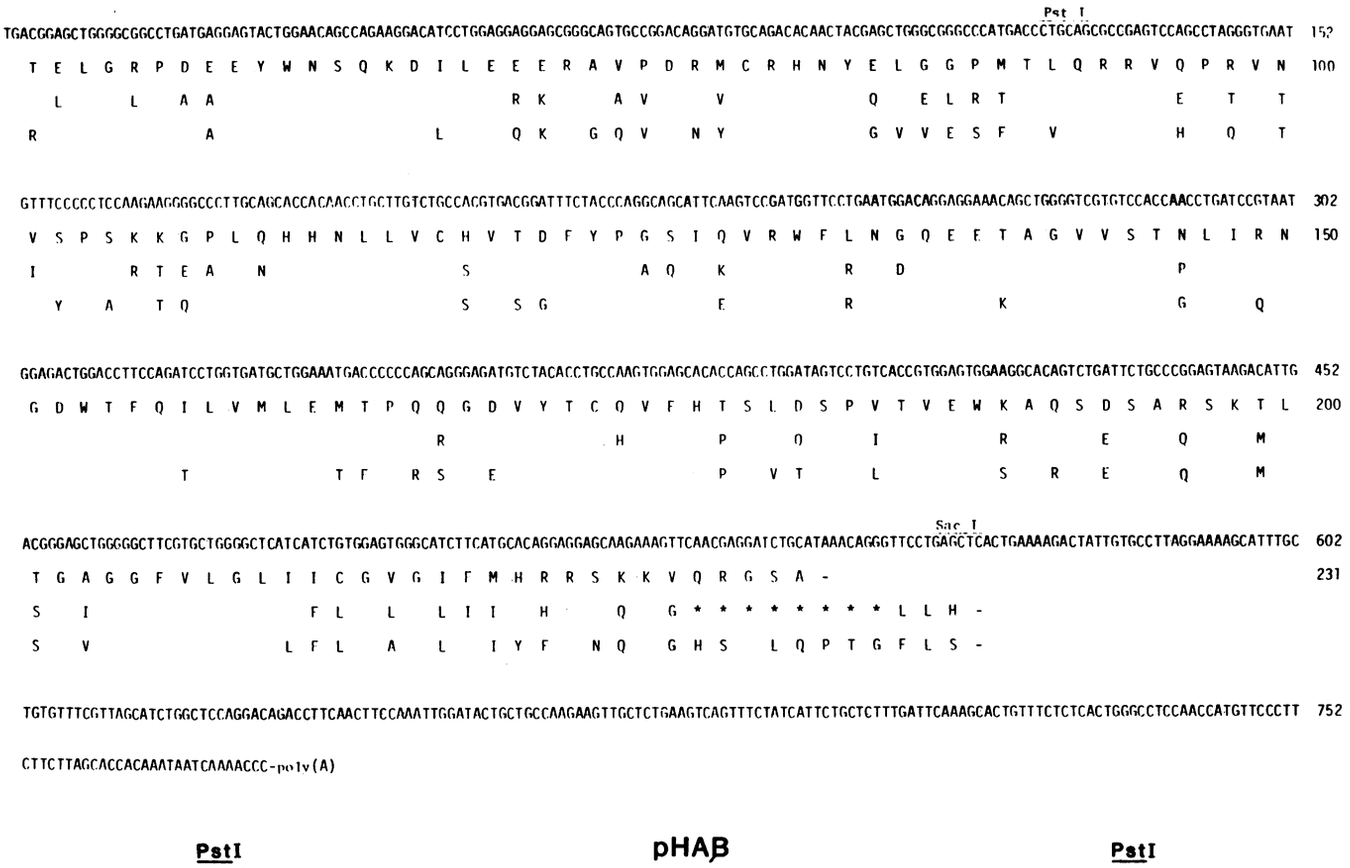
The second external domain (β2) of class II β chains has been shown to be relatively conserved and to share significant amino acid sequence homology with Ig constant domains as well as with $\beta_2$-microglobulin and the α3 domain of the class I HLA heavy chain (24–26, 28, 29). Sequence comparison of the HA β2 domain to the constant domain of immunoglobulins displayed 17 shared residues distributed throughout the two sequences. The same 17 residues have been found shared with the third extracellular domain of class I antigen heavy chains (30), $\beta_2$-microglobulin (31), and the second extracellular domain of class II antigen α chains (15, 32).

The comparison of DR or DC β2 domains with the β2 domain of HA revealed an overall homology of 72% or 77%, respectively. Conspicuously, a stretch of 20 amino acid residues (positions 147–166) displayed 100% homology between the HAβ and the DCβ sequences, whereas it exhibited limited conservation when compared to its DRβ counterpart (75%). DNA sequence comparisons of HAβ and DCβ showed 88% homology within this segment, and the homology was 83% when either HAβ or DCβ was compared to the corresponding fragment of a mouse *I-Aβ* gene (16). In contrast, the homology found between DRβ and *I-Aβ* was 73%. These results suggest that the *HAβ* locus is more closely related to an *I-A*-like locus than *DRβ* and predict a common ancestor to *HAβ* and *DCβ* genes (compare ref. 14).

A separate exon encodes the connecting peptide, the transmembrane region, and the intracellular portion of class II antigen β chains in mouse and man (ref. 16; unpublished data). Only a few residues of this exon were conserved in HAβ, DRβ, and DCβ sequences. These domains may be locus-specific (14, 15), and multiple substitutions in these regions of the molecules may be designated for specific interactions with either the corresponding α chains or particular molecules in the cytoplasm of the cell.

T-cell activation in response to a particular foreign antigen is determined by the repertoire of cell-surface histocompatibility antigens (33) accounting for the extreme polymorphism of some MHC antigens and suggesting a selective advantage for heterozygosity. Multiple, closely related and expressed β-chain genes within the *D* region could enhance HLA heterozygosity and increase the number of possible contexts in which a foreign antigen would be presented. Moreover, association of different β chains to the same α chain has been described (1). Such association with *cis*- or *trans*-linked (or both) α chain gene products could contribute to a dramatic increase of the MHC repertoire displayed by an individual.

**Multiplicity and Polymorphism of Genes Encoding the HAβ Chain.** Several lines of evidence suggest that the *D* region is complex and encodes multiple α and β chains at both the *DR* and *DC* loci (1). Furthermore, functional analyses predict that loci encoding class II antigens, in addition to *SB* (3–9), might be located within the region defined by recombination and centromeric to *DR* (unpublished data). To answer the question of how many genes hybridize to the pHAβ probe, genomic DNA prepared from 5 HLA-DR homozygous B-cell lines and PBL from 16 unrelated individuals typed for HLA antigens was digested with the restriction endonuclease *Hin*dIII. Genomic fragments of 8.3, 5.6 or 6.0, and 1.9 kb hybridized to the HAβ probe under highly stringent conditions (Fig. 2 and Table 1). Because *Hin*dIII does not cut the HAβ cDNA, these results suggest either that there are multiple *HAβ*-related genes in the human genome or that multiple *Hin*dIII sites are located within introns in a unique *HAβ* gene. The pattern of hybridization obtained with a probe corresponding to the β1 domain (pHAβ-2) and the 3′-untranslated region (pHAβ-15) was found to be indistinguishable from that obtained with a probe containing the $\beta_2$ domain through the 3′-untranslated region (pHAβ-10). This



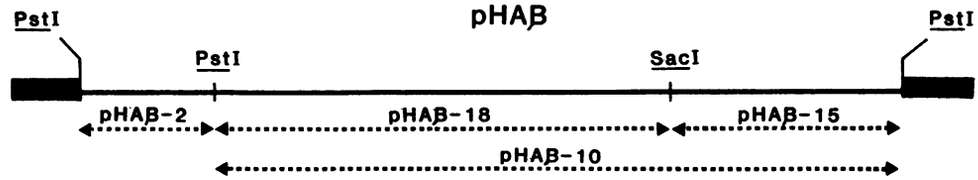




FIG. 1. Nucleotide sequence of the pHA$\beta$ cDNA. DNA sequence and nucleotide number of the pHA$\beta$ cDNA are indicated on the first line. The amino acid translation of the nucleotide sequence is shown on the second line; the deduced amino acid sequence of a DC$\beta$ (24) and a DR$\beta$ chain (25) are shown on the third and the fourth lines. Only those amino acid residues that are different from the HA$\beta$ sequence are shown. Amino acid sequences within the cytoplasmic region were aligned with respect to the *I-A*$\beta$ gene sequence (16). Accordingly, eight amino acids were found missing in the DC$\beta$ sequence (*). The cytoplasmic region of HA$\beta$ is six amino acids shorter than the corresponding region of DR$\beta$. Nevertheless, the 18 nucleotides following the stop codon in the HA$\beta$ sequence display high conservation with the DNA sequence coding for the last six amino acids of the DR$\beta$ carboxyl terminus. Amino acid positions were indicated with respect to the complete sequences (24, 25). The restriction sites used for subcloning and the subclones obtained (see text) are indicated.

result suggests that there are at least two (and possibly three) genes with sequences highly homologous to pHA$\beta$ in the human genome. Moreover, the DNA polymorphism detected in the 5.6- to 6.0-kb range (Fig. 2 and Table 1) is consistent with heterogeneity introduced by allelic differences. This restriction fragment polymorphism was also seen with *Eco*RI (see below).

Because the observed variation did not segregate with either DR or DC specificities, the DR homozygous cell lines were typed for SB antigens. Four cell lines were homozygous at the *SB* locus, either SB2 or SB4, whereas the fifth was heterozygous SB3,4. This analysis was also extended to 16 individuals typed for all HLA antigens (Table 1). The 6.0-kb fragment correlated with SB4; in addition, the one SB1 individual and the one SB5 individual also had this fragment. The 5.6-kb DNA fragment correlated with SB2 or SB3. The SB4 specificity is the most common in the population, and it can probably be split into subtypes (34, 35). Therefore, it is interesting to note that one individual who typed as SB2,4 did not have the 6.0-kb fragment. This specificity has been designated SB4.2 to distinguish it from the most prominent SB4.1 category (11 individuals). Therefore, these results are consistent with the existence of a genetic linkage between the pHA$\beta$ clone, the 5.6- to 6.0-kb polymorphic DNA fragments, and the *SB* locus.

**Genetic Independence of the *HA*$\beta$ Locus.** Lymphoblastoid cell lines that are hemizygous for *HLA* loci have been created by using $\gamma$-rays to generate deletions in single haplotypes of the heterozygous cell line LCL721 (9, 20). The introduction of additional $\gamma$-ray-induced deletions generates various homozygous deletion mutants (i.e., mutants in which both genes at a given locus are deleted). Such mutants were used to resolve the expression of DR and DC/MT or DR and SB (9, 10). To map the *HA*$\beta$ locus with respect to *DR*, *DC/MT*, and *SB* loci, DNA of several deletion mutants was analyzed by blot hybridization with the pHA$\beta$-10 probe. All variants used here have lost expression of DR1 and some have lost DR1 and DC1/MT1, but all have maintained expression of *cis*-linked SB2 (see legend to Fig. 3). *Hin*dIII DNA fragments of 1.9, 5.6, and 8.3 kb were detected in all deletion mutants, whatever their phenotype (Fig. 3*A*)—i.e., DR-null (lanes 1 and 2) or DR-null, DC/MT-null (lanes 3–5). Furthermore, these patterns are consistent with the SB2 typing of these variants. One additional 3.4-kb DNA fragment was detected in the 721.82.4 mutant (Fig. 3*A*, lane 5).

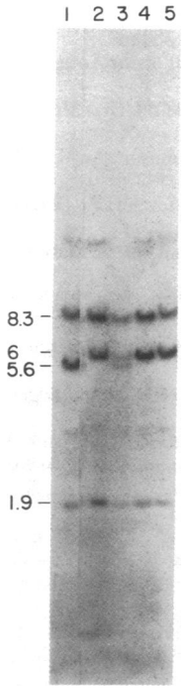



FIG. 2. pHA$\beta$-10 probe hybridized to digested DR homozygous human B-cell line DNAs. Genomic DNA was digested with a 5-fold excess of *Hin*dIII, displayed on a 0.7% agarose gel (10 $\mu$g per slot), and transferred to nitrocellulose filters. The filters were prehybridized, hybridized with $^{32}$P-labeled pHA$\beta$-10, and washed under high stringency conditions. Lanes 1–5: WT46 (DRw6, SB2), Mich (DR5,SB4), Priess (DR4,SB3,4), WT49 (DR3, SB4), and PGF (DR2, SB4). Molecular sizes (in kb) were estimated by comparison to $\lambda$-*Hin*dIII markers.

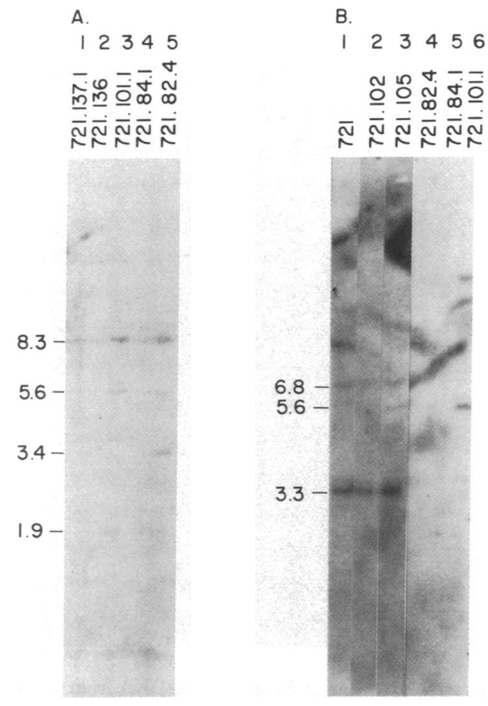



FIG. 3. Hybridization of HA$\beta$ (*A*) and DC$\beta$ (*B*) probes to DNA from $\gamma$-ray-induced deletion mutants. HLA typing: 721.102, -.105, -.136, and -.137.1: A2, B5, DR-null, MT1, SB2; and 721.82.4, -.84.1, and -.101.1: A2, B5, DR-null, MT-null, and SB2. Ten micrograms of each digested DNA was used as described in the legend to Fig. 2. Restriction fragments from parental cell line (lane 1 in *B*), and SB$^+$, DC$^+$/MT$^+$, DR-null mutants (lanes 1 and 2 in *A* and lanes 2 and 3 in *B*), SB$^+$, DC/MT-null, DR-null mutants (lanes 3–5 in *A* and lanes 4–6 in *B*) were visualized by hybridization with the pHA$\beta$-10 (*A*) or pDC-$\beta$1 (*B*) probes after low stringency washing conditions. Fragment sizes (kb) were estimated from the *Hin*dIII digest of $\lambda$ DNA run in a parallel channel.

Because of the closer relatedness between HA$\beta$ and DC$\beta$ than HA$\beta$ and DR$\beta$ (see above), specific patterns generated with a DC$\beta$ probe were investigated (24). Two fragments of 6.8 and 3.3 kb hybridized with the DC$\beta$ probe in the *Hin*dIII-restricted DNA prepared from the LCL721 parental cell line (Fig. 3*B*, lane 1) and its DR-null variants (Fig. 3*B*, lanes 2 and 3). In contrast, these two bands were missing in DNA from DR-null, DC/MT-null variants (Fig. 3*B*, lanes 4–6). An additional fragment of 5.6 kb was detected in a DR,DC/MT homozygous deletion mutant (Fig. 3*B*, lane 6), suggesting that partial deletion or rearrangement events occurred at the *DC$\beta$* locus during the generation of this particular variant.

The results presented here clearly point out that the *HA$\beta$* locus is distinct from the *DR$\beta$* and *DC$\beta$* loci. Nevertheless, because mutants that are DR$^+$, DC$^+$/MT$^+$, SB-null are not presently available, it is not possible to firmly assign the *HA$\beta$* gene to the *SB* locus from these data.

**Definitive Assignment of the *HA$\beta$* Gene(s) to the *SB* Region.** Previous family studies were reported to show recombination between *SB* and *DR* (3–8). In family Fa(3), a recombination divided the *HLA-D* region into two subregions, a telomeric region, which includes *DR* as well as *DC/MT*, and a centromeric region, which includes *SB* (8). Siblings G (*b/d*) and I (the recombinant *b'/d*, where *b'* = *b/a*), who have inherited all of the HLA A,B,DR and MT markers of the *b* haplotype and the same maternal *d* haplotype, differ only for one allele at the *SB* locus (see legend to Fig. 4). Therefore, any DNA polymorphism detected with a specific MHC probe would map the corresponding sequence to the *SB* subregion. High molecular weight DNA from individuals G and I was digested with seven restriction endonucleases and analyzed by blot hybridization with pHA$\beta$-10 under conditions that allow the detection of some cross-hybridizing sequences (i.e., *DC$\beta$*- and *DR$\beta$*-like sequences). Two enzymes clearly detected a DNA polymorphism between the two individuals: *Eco*RI (Fig. 4, lane 3) and *Hin*dIII (lane 4). The *Hin*dIII polymorphism corresponds exactly to the 5.6- to 6.0-kb variation described above. Therefore, this experiment clearly maps the polymorphic DNA fragments to the *SB* region, which accordingly contains one or more *HA$\beta$*-related genes.

Table 1. Correlation of *SB* types with *Hin*dIII restriction fragments in 16 individuals

| | | SB2 or SB3 | | | | SB4 | |
|---|---|---|---|---|---|---|---|
| | | + | − | | | + | − |
| 5.6-kb fragment | + | 12 | 0 | 6.0-kb fragment | + | 11 | 2* |
| | − | 0 | 4 | | − | 1† | 2 |

Genomic DNA from 16 individuals was digested with restriction endonuclease *Hin*dIII. Southern blotting analysis of the DNA digests after electrophoresis showed that the pHA$\beta$ probe reassociated with either three or four digest fragments in each individual (see Fig. 2). Fragments of 8.3 and 1.9 kb were present in every individual. The presence of the 5.6-kb and 6.0-kb fragments was correlated with the *SB* allele(s) present in an individual.

* The 6.0-kb fragment was also present in one SB1 individual and one SB5 individual, who were SB4 negative.

† SB4 is a complex specificity (34, 35). This individual may be an example of an SB4.2 specificity.

**Conclusion and Prospective.** Because of their role in the control of the immune response, the structure of the multiple human class II antigens is of special interest. DR and DC antigens have been shown to be the human counterparts of murine I-E and I-A antigens, which have been extensively studied functionally. Although the genetic organization of the murine *I* region (where the *I-A* and *I-E* subregions have been located) appears relatively simple (36), its human equivalent, the *D* region, is much more complex as already evident in studies of the class II $\alpha$-chain genes (37, 38). In this paper, a cDNA clone,

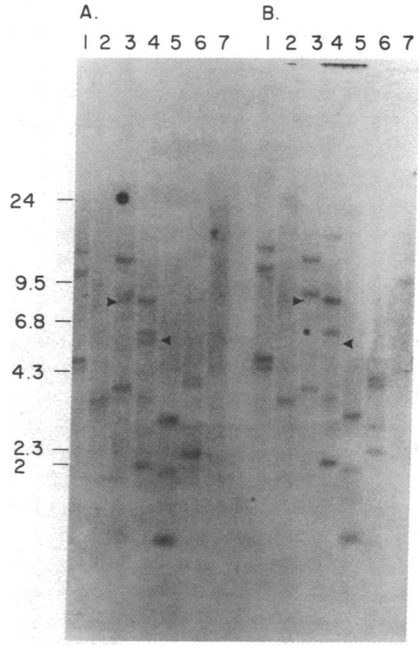



FIG. 4. Hybridization of HA$\beta$ probe to DNA from sibs that inherited the original (sib G, *b/d*) or a recombinant (sib I, *b'/d*) paternal *b* haplotype (3, 4, 8). HLA haplotypes: *a*, Aw24 B14 DW3 DR3 MT2 SB1 GLO2; *b*, A11 B35 DW6 DR6 MT1 SBblank GLO1; *b'* = *b/a*, A11 B35 DW6 DR6 MT1 SB1 GLO2; *d*, A30 Bw16 DW6 DRw6 MT1 SBblank GLO1. Ten micrograms of G (*A*) and I (*B*) DNAs was digested with *Bam*HI (lane 1), *Bgl* II (lane 2), *Eco*RI (lane 3), *Hin*dIII (lane 4), *Pst* I (lane 5), *Pvu* II (lane 6), and *Sma* I (lane 7) and displayed as mentioned in the legend to Fig. 2. The filters were then hybridized with $^{32}$P-labeled pHA$\beta$-10 probe. The filters were washed under low stringency conditions. The arrows indicate fragments that point out the SB1/SBblank polymorphism between the two haplotypes. The size in kb of $\lambda$ *Hin*dIII restriction fragments is indicated on the left.

pHA$\beta$, corresponding to a third isotypic form of human class II $\beta$ chain is described by DNA sequence analysis and study of $\gamma$-ray-induced deletion variants of a human B-cell line. Polymorphic DNA fragments that correlated with distinct *SB* genotypes within a panel of unrelated individuals, as well as *SB* allelic variations between two DR,DC/MT identical sibs in an informative family, allowed assignment of the *HA*$\beta$ locus to the *SB* region of the *D* complex.

Expression of the *DR*, *DC*, and *SB* $\alpha$ and $\beta$ chain genes in various cell types will allow the establishment of direct correlations between particular genes and serologically or functionally defined specificities and will make possible an investigation of the nature of the functional difference existing between these three subsets of human class II antigens.

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