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Immunology

Gene organization of DC and DX subregions of the human major histocompatibility complex

(gene sequences/promoters/DNA·DNA hybridization/HLA antigens)

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ABSTRACT **The DC and DX subregions of the human major histocompatibility complex (MHC) have been cloned from a cosmid library made from a human B-cell line, Priess. The DC subregion, 48 kilobases, includes the *DCα* and *DCβ* genes. A second DC-like region, the DX subregion, 35 kilobases, contains the *DXα* gene and a newly found *β* gene termed *DXβ*. Since the DC and DX genes are highly homologous in nucleotide sequence, gene size, exon–intron organization, and direction of transcription, the DC and DX subregions were presumably generated by duplication of an ancestral *α–β* gene pair. Nucleotide sequencing indicates that all four genes have intact coding sequences and promoter regions. Homology between the upstream promoter sequences of these four genes and seven other class II genes at nucleotides −69 to −78 and −98 to −110 highlights these previously described conserved elements. Moreover, a striking conservation of flanking *α*-gene-specific and *β*-gene-specific sequences has been observed. Comparison of Southern blots of Priess DNA with *DCα* and *DCβ* cDNA probes with isolated cosmid clones showed that (*i*) the human chromosome encodes only two *DCα*-related and two *DCβ*-related genes, namely, *DCα*, *DXα*, *DCβ*, and *DXβ*, and (*ii*) the DC and DX subregions are homozygous in Priess cells.**

The class II antigens of the major histocompatibility complex (MHC) are transmembrane glycoproteins consisting of an *α* subunit and *β* subunit. The antigens are expressed on the surface of B cells, some T cells, and macrophages, and they function in regulation of the immune response and in communication between lymphocytes and are encoded in three subregions, DR, DC, and SB (1–5). The SB subregion of about 100 kilobases (kb) has recently been shown to have two *α* and two *β* genes in the order *SBα–SBβ–SXα–SXβ* (6, 7). However, the genetic organization of class II genes in the rest of the HLA-D region is not yet well understood. Study of the DC subregion was initiated by isolation of *DCα* (8) and *DCβ* cDNA clones (9), followed by isolation and sequencing of the corresponding genomic clones (10–12). When the isolated cDNA clones were used as probes, Southern blots of DNA from a homozygous cell line indicated the presence of two closely related *α* gene bands, named *DCα* and *DXα* (4, 13–15) and two (or more) *β* gene bands (12, 16–19). A genomic clone for *DXα* was also isolated and sequenced (10).

To elucidate further the gene organization of the DC subregion, a genomic library from a typed human B-lymphoblastoid cell line, Priess (DR4/4, DC4/4, SB3/4), was screened with *DCα* and *DCβ* cDNA as probes. Several cosmid clones that carry both the *DCα* and *DCβ* genes were isolated, as well as clones encoding *DXα* and a second *β* gene, termed *DXβ*. In this paper, physical maps of the DC and DX subregions containing these genes are presented and some features of their sequences are compared. In addition, Southern blots of Priess cell DNA were compared with the isolated clones to identify the genes.

MATERIALS AND METHODS

Screening of the Genomic Library. A cosmid library was constructed from DNA prepared from a human B-lymphoblastoid cell line, Priess (20, 21), which was partially digested with *Mbo* I and then ligated to the cosmid vectors pTCF and pGNC (22). Clones were screened by colony hybridization using nick-translated *DCα* or *DCβ* cDNA as probes. Detailed procedures for library construction and screening of clones are described elsewhere (7).

cDNA Clones Used as Probes. The *DC4α* cDNA clone, pDCH1 (8), contains the α_1, α_2, transmembrane (TM), and 3′ untranslated (3′ UT) exons, while the *DC1α* cDNA clone, HB20 (10), is full length. The *DC1β* cDNA clone, DK30 (D. Kappes, personal communication), includes part of the β_1 exon and the entire sequence of the β_2, TM, and 3′ UT exons.

Restriction Site Mapping and Blot Hybridization. Restriction maps were made by digestion of cosmid and λ phage clones with one or a combination of restriction enzymes and by blot hybridization of the digested fragments with cDNA probes (7). Southern blotting was carried out as previously described (7, 23).

Sequencing. The cosmid clone B20A was subcloned in the vector pUC13 for sequence analysis using the chemical degradation method of Maxam and Gilbert (24). Sequences of both strands were obtained.

RESULTS

Isolation of Cosmid Clones. Cosmid libraries of about one million clones, prepared from the Priess cell line as previously described (7), were screened by colony filter hybridization with nick-translated cDNA fragments of DC*α* and DC*β*. Positive clones were isolated and analyzed by blot hybridization using a set of cDNA clones derived from the *α* and *β* genes of the three known subtypes, DR, DC, and SB. The clones that showed strongest hybridization with the *DCα* or *DCβ* probe under stringent washing conditions were tentatively considered to be DC clones. Ten clones of this type were isolated and separated into two groups from restriction mapping and blotting data. One group of eight clones (H11A, T10F, T28L, T10E, G8B, G5-5, H19A, and X10A) covers a 48-kb length and encodes one *α* and one *β* gene (Fig. 1*A*). The other group of two clones, H15A and B20A, spans a DNA stretch of 35 kb and has one *α* and one *β* gene (Fig. 1*B*). The two groups do not overlap with each other.

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Abbreviations: MHC, major histocompatibility complex; kb, kilobase(s).

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Identification of the α Genes. Several investigators have reported the presence of two DC-related α genes, *DCα* and *DXα*, in homozygous human cell lines (4, 13–15). Blot analyses of Priess DNA with the *DCα* cDNA fragment as a probe showed two *Hin*dIII fragments reported as 5.5 and 2.6 kb (4) but estimated as 4.9 and 2.5 kb in the present study. The *Eco*RI fragments were 6.3 and 5.1 kb (Fig. 2*A*). The 4.9-kb *Hin*dIII fragment corresponds to the *DCα* gene and the 2.5-kb *Hin*dIII fragment corresponds to the *DXα* gene (4, 13). The eight cosmid clones of the first group (Fig. 1*A*) have the 4.9-kb *Hin*dIII fragment and the 6.3-kb *Eco*RI fragment, which hybridize with the *DCα* probe, whereas the two clones of the second group (Fig. 1*B*) have the 2.5-kb *Hin*dIII and the 5.1-kb *Eco*RI fragments (Fig. 3*A*). Therefore, the clones of the first group must have the *DCα* gene, and those of the second group, the *DXα* gene. Further identification of the genes was done by comparing detailed restriction maps with those derived from the nucleotide sequences of the *DCα* and *DXα* genes (10). The restriction map from the sequenced *DC4α* (λDCH-9 clone) and *DXα* (λDCH-10 clone) genes agreed with restriction maps of the cosmid clones of the first and the second groups, respectively.

Blot hybridization of five human class II α genes with the *DCα* cDNA probe under relaxed and stringent washing conditions showed that both the *DCα* and *DXα* genes hybridized much more strongly than the *SBα*, *SXα*, and *DRα* genes, indicating that the *DXα* gene is highly homologous to the *DCα* gene, particularly in the restriction fragment containing the α_2 exon (5.1-kb *Eco*RI fragment) (Fig. 4).

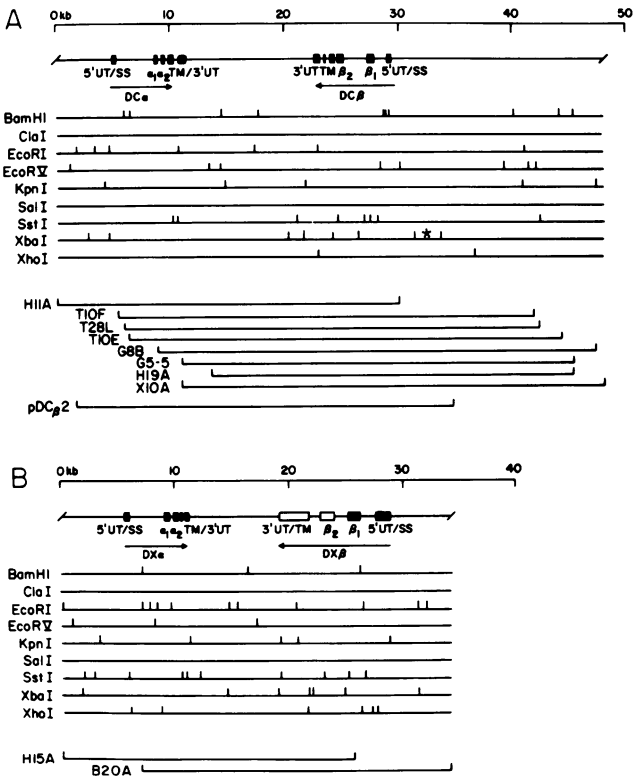

FIG. 1. Molecular maps of the DC subregion (*A*) and the DX subregion (*B*). The length of the region (kb) is shown on the top line. On the second line, the α and β genes are mapped. Solid boxes indicate exons. For the *DXβ* gene, approximate sites for exons 3–5 are shown by open boxes. Arrows indicate directions of transcription 5′ → 3′. Restriction sites for nine enzymes (*Hin*dIII, *Cla* I, *Eco*RI, *Eco*RV, *Kpn* I, *Sal* I, *Sst* I, *Xba* I, and *Xho* I) are shown by vertical bars. At the DNA region marked *, three small *Xba* I-digested fragments, 1.1, 0.6, and 0.5 kb, were mapped. Their order is unknown. Overlapping cosmid clones are given below the restriction maps.

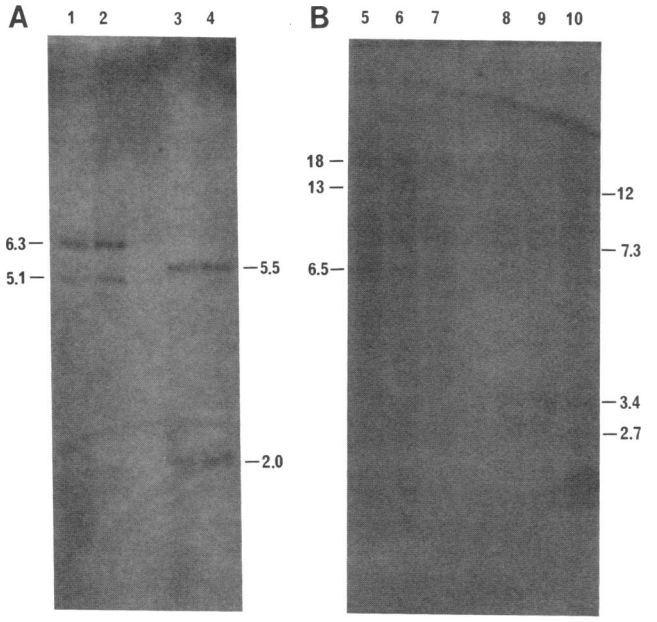

FIG. 2. Southern blot hybridizations. Genomic DNA from cell lines Priess (DR4/4, DC4/4, SB3/4, our stock) (lanes 1, 3, 6, and 8), Priess (provided by J. Bodmer) (lanes 2, 4, 7, and 9), and WT49 (DR3/3, DC3/3, SB4/4) (lanes 5 and 10) was digested with *Eco*RI (lanes 1, 2, and 5–7), *Taq* I (lanes 3 and 4), or *Hin*dIII (lanes 8–10). The filters were hybridized with nick-translated *DCα* cDNA fragment (HB20) (*A*, lanes 1–4) or *DCβ* cDNA fragment (*B*, lanes 5–10) and washed at 65°C. with 0.5× NaCl/Cit containing 0.05% $NaDodSO_4$. Fragment sizes are shown in kb.

Identification of the β Genes. The cosmid clones of both groups have one β gene as well as one α gene, as shown in blots with the *DCβ* cDNA probe (Fig. 3*B*). The β gene linked with the *DCα* gene on clones of the first group was named *DCβ*, and the β gene linked with the *DXα* gene on clones of the second group, *DXβ* (Fig. 1). *DCβ* and *DXβ* genes hybridized with the *DCβ* cDNA probe much more strongly than the other known human class II β genes, *DRβ*, *SBβ*, and *SXβ* (data not shown but similar to Fig. 4).

Genomic blots using DNA from homozygous cell lines show two to four prominent bands that hybridize with *DCβ* cDNA (12, 16–19). In the case of Priess DNA, two *Eco*RI bands, 18 and 6.5 kb, were detected (12, Fig. 2*B*). The 18-kb

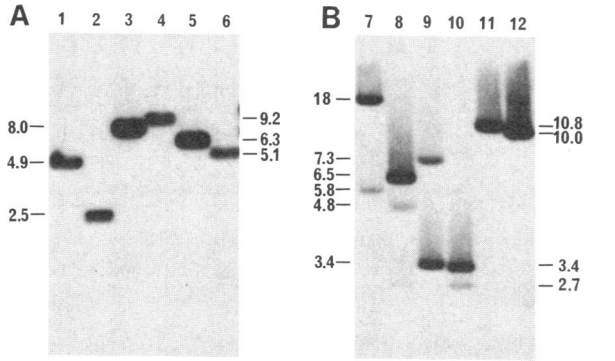

FIG. 3. Blot hybridization of DC and DX cosmid clones. Clones were digested with *Hin*dIII (lanes 1, 2, 9, and 10), *Bam*HI (lanes 3, 4, 11, and 12), or *Eco*RI (lanes 5, 6, 7, and 8). The filters were hybridized with nick-translated *DCα* cDNA fragment (HB20) (*A*, lanes 1–6) or *DCβ* cDNA fragment (*B*, lanes 7–12) and washed at 65°C with 0.1× NaCl/Cit containing 0.05% $NaDodSO_4$. Lanes 1, 3, and 5, H11A clone, which has an intact *DCα* gene; lanes 2, 4, and 6, H15A clone (*DXα*); lanes 7, 9, and 11, T28L clone (*DCβ*); lanes 8, 10, and 12, B20A clone (*DXβ*).

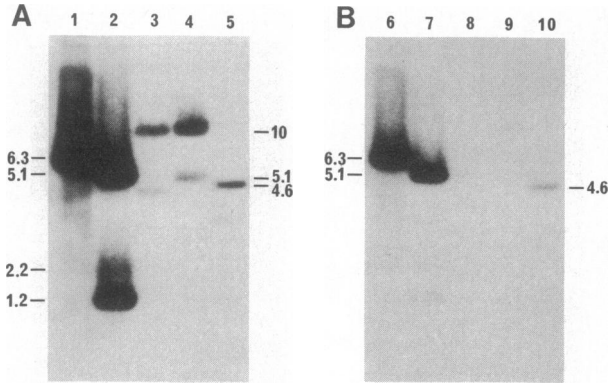

FIG. 4. Blot hybridization of clones carrying α genes of different HLA-D region subtypes. Clones were digested with *Eco*RI, hybridized with labeled *DC*α cDNA fragment (HB20), and washed at 65°C with 3× NaCl/Cit containing 0.05% $NaDodSO_4$. After the autoradiogram (*A*) had been made, the filter was extensively washed at 65°C with 0.1× NaCl/Cit (*B*). Lanes 1 and 6, H11A clone, which has a *DC*α gene; lanes 2 and 7, H15A clone (*DX*α gene); lanes 3 and 8, T10B clone (*SB4*α and *SX4*α genes); lanes 4 and 9, G8A clone (*SB3*α and *SX3*α genes); lanes 5 and 10, T9C clone (*DR4*α gene). All the clones were isolated from a cosmid library made from Priess cell DNA. In lanes 3 and 4, the strong bands at 10 kb have the *SB*α gene, and the weak bands at about 4.5 and 5.1 kb have the *SX*α gene (7).

fragment and 6.5-kb fragment correspond to the *DC*β and *DX*β genes, respectively. A weak 12-kb band seen in an earlier study (12) is probably a partial digestion product composed of the major 6.5-kb and minor 4.8-kb bands of the *DX*β gene (Figs. 1 and 3*B*).

A DCβ gene isolated from DNA of a homozygous human individual typed as DR4/4, the same haplotype as Priess, has been sequenced (11). Restriction enzyme maps of this β gene are exactly the same as the maps of the *DC*β gene shown in Fig. 1*A* and quite different from the *DC*β gene from a DR 3/3 cell (12).

Gene Organization. The locations of each exon in all four genes were elucidated from blots using probes for each of the exons individually. Solid boxes in Fig. 1 show the exons of *DC*α, *DC*β, and *DX*α genes and some *DX*β exons. Open boxes indicate the approximate locations of the other *DX*β gene exons. The DC and DX regions have several common features of gene organization: (*i*) the *DC*α and *DX*α genes each encompass about 6 kb, with long introns between the 5′UT/SS and α_1 exons. (*ii*) The *DC*β and *DX*β genes are bigger than the α genes (7–10 kb). (*iii*) *DC*α and *DC*β genes are linked in a tail-to-tail (3′ end to 3′ end) manner at a distance of 12 kb. (*iv*) *DX*α and *DX*β genes are similarly oriented (tail-to-tail) at a distance of about 8 kb.

A cosmid clone, pDCβ2, that has *DC*α and *DC*β genes has been isolated from another cell line, JY (DR4/6, DC4/1). Physical maps with several restriction enzymes and blots with *DC*α and *DC*β cDNA probes showed that the cloned region of the pDCβ2 clone is identical to the clones isolated from Priess cells and, therefore, is derived from the DR4, DC4 haplotype (Fig. 1). In addition, it is the only one of the cosmids that contains both an intact α and an intact β gene. Preliminary experiments indicate that the DC4 antigen is expressed at the surface when this cosmid is transfected into mouse Ltk^- cells (unpublished results).

Sequences of the Two α and Two β Genes in the DC and DX Clusters. The sequence information for three of the genes in this cluster has already been published, for both cDNA and genomic clones of *DC*α and *DC*β (8–12) and for a genomic clone of *DX*α (10) (for which no cDNA clone has so far been identified). Of particular interest is the question of whether the *DX*α and *DX*β genes are intact and expressed genes. The sequence information already published for *DX*α indicates that its α_1, α_2, transmembrane–intracytoplasmic, and 3′ untranslated exons are intact (10). Moreover, insertion of this gene into a retrovirus vector followed by transfection into psi2 cells followed by virus harvest and infection of NIH 3T3 cells has indicated that this gene appears to be spliced normally in these cells (A. Korman, personal communication). Some information has also been obtained for the sequence of the *DX*β gene (Fig. 5). This gene also contains an intact β1 exon, which has 83% nucleotide sequence homology to *DC*β and 74% amino acid homology (23 substitutions out of 88 residues, including a single amino acid deletion in *DX*β). A much higher degree of divergence is found when comparing this sequence to *DR*β (55%, 40 substitutions out of 88 residues) or *SB*β (56%, 39 out of 88, including two deletions in *SB*β). Thus, the sequence data confirm the blot hybridization in indicating that *DX*β is much more closely related to *DC*β than to *DR*β or to *SB*β. Moreover, *DX*α and *DC*α are similarly closely related (20 substitutions out of 83 residues). In addition, the β_2, transmembrane–intracytoplasmic, and 3′ untranslated exons of a *DX*β gene from another DR4 homozygous cell have been sequenced by D. Larhammar (personal communication), and these domains also appear to be intact. Thus, like *DX*α, the *DX*β gene appears to be intact in its coding sequences.

Promoter and Signal Sequences of the Two α and Two β Chain Genes in the DX and DC Cosmids. Among these four

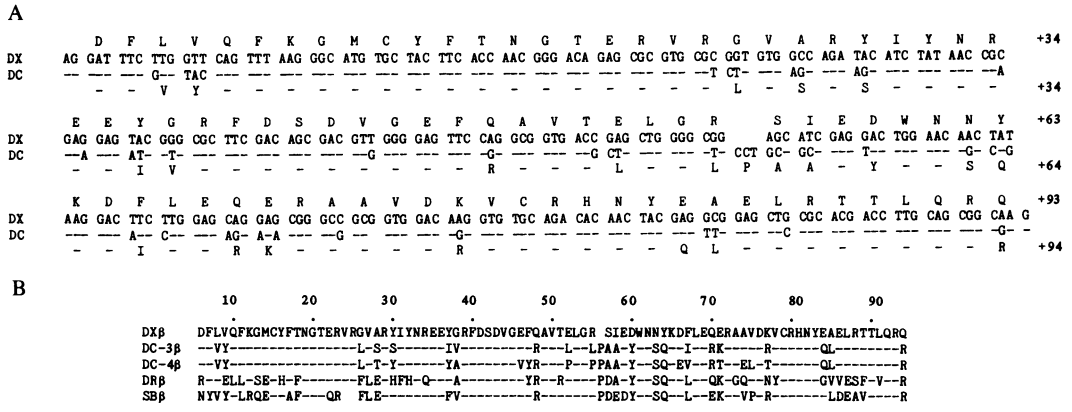

FIG. 5. Nucleotide and amino acid sequences of the β_1 exon of the *DX*β gene. (*A*) The nucleotide sequences of the *DX*β and *DC3*β chain genes are compared. Corresponding amino acids (standard one-letter code) are also compared. Dashes indicate positions where the sequences of the genes are identical, and amino acid numbers are shown on the right. (*B*) The amino acid sequence of the β_1 domain of the *DX*β gene is compared to the sequences of the β_1 exons of *DC3*β (12), *DC4*β (11), *DR*β pseudogene (25), and *SB*β (26). Dashes indicate identical residues, and amino acid numbers are shown above.

genes, so far only the promoter region and signal sequences of the *DCβ* gene have been published (11, 12). These sequences for the other three genes have also been obtained. Little homology exists among them in the region immediately upstream of the transcription initiation site—i.e., in the "TATA" and C-C-A-A-T region. However, further upstream a region of strong homology is found that was observed earlier in comparing *I-Eα* with *DRα* (27). Later addition of a third sequence (*I-Eβ*) allowed the definition of the conserved region (28). The sequences in this region for *DCα*, *DXα*, *DCβ*, and *DXβ* are presented in Fig. 6 and compared to published sequences for *I-Aβ* (29), *I-Eβ* (28), *SBβ* (26), a *DRβ* pseudogene (25), *DRα* (30), and *I-Eα* (31). The compilation of these sequences indicates, first of all, that the conserved sequences at −69 to −78 and −98 to −110 are also present in all four genes in the DC–DX clusters. Second, this compilation of sequences from 11 different genes firmly establishes the occurrence of these upstream conserved sequences in all members of this gene family and strongly suggests a role for these sequences in their expression. The two sequences are separated by 20 nucleotides in the case of the α genes and 19 nucleotides in the case of the β genes (with the exception of *SBβ* which has only 18 nucleotides).

Particularly striking, in addition, is the conservation of α-gene-specific or β-gene-specific sequences surrounding the −69 to −78 element. In the case of the β chain genes a conserved pyrimidine element, C-Y-Y-Y, occurs just downstream at nucleotides −68 to −65, while in the α chain genes this element is all purines, A-A-A-R. Just upstream at −82 to −79, A-R-T-G occurs in all of the β chain gene sequences, while in the same region at −84 to −79 the absolutely conserved A-T-T-T-T-T occurs in all of the α chain gene sequences. Other conserved nucleotides specific either to α or to β chain gene sequences occur in the 19 or 20 nucleotides that separate the −110 to −98 and −78 to −69 sequences, as is evident from inspection of the consensus sequences (Fig. 6). Particularly striking is the conservation of all 19 nucleotides at −79 to −97 among *DC3β*, *DC4β*, and *DXβ* and, similarly, conservation of 19 out of 20 nucleotides in this region between *DC4α* and *DXα*. This region is much more conserved than are the −98 to −110 elements of these genes. The occurrence of α-chain-specific and β-chain-specific sequences suggests that these elements could be important in the coordinate regulation of α and β chain gene transcription. They may also be involved in control of the rate of transcription; i.e., all of these genes have minor variations in sequences in these upstream regions and all are known to be expressed to different extents.

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                -110          -98                    -78      -69
Consensus α and β CCYAGNRACNGATG                      CTGATTGGYY
          DC-3β  --C--AG--A---G  AGGTCCTTCAGCTCCAGTG  --------TT CCTT
          DC-4β  --T--AG--A---T  AGGTCCTTCAGCTCCAGTG  --------TT CCTT
          DXβ    --C--AGG-A---G  AGGTCCTTCAGCTCCAGTG  --------TT CCTT
          I-Aβ   --C--AG--A---G  ACAGACTTCAGGTCCAATG  --------TT CCTC

          SBβ    --T--TG-GCA--G  CTCATACAAAGCTC AGTG  TCC-----TT CTTT
          I-Eβ   A-T--CA--T---G  ATGCTGGACTCCTTTGATG  --------CT CCCA
          DRβ    A-C--CA--T---G  ATGCTATTGAACTCAGACG  ------CATT CTCC

Consensus β only                 ANRYYNYYCAGCTCCARTG             CYYY


          DCα    G-T--TA--T--GA TGTCACCATGGGGG ATTTTT --A-----CC AAAA
          DXα    A-TG-CA-ACA--A TGTCACCATAGGGG ATTTTT --------CC AAAA
          DRα    --T--CA--A---G CGTCA TCTCAAAATATTTTT --------CC AAAG
          I-Eα   --T--CA--A---G TGTCAGTCT GAAACATTTTT --------TT AAAA

Consensus α only                 TGTCANYCTNRRRRNATTTTT            AAAA
                                           A
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FIG. 6. Comparison of the upstream sequences common to the class II MHC genes. The *DC4α*, *DXα*, and *DXβ* sequences were determined in this study. References to sequences of other genes are given in the text. The numbering is nucleotides upstream from the initiation of transcription of *DC3β*. Spaces are placed where needed within the sequences to maximize homology. Y, pyrimidine; R, purine; and N, any nucleotide.

In addition, all four genes, *DCα*, *DCβ*, *DXα*, and *DXβ*, encode leader sequences beginning with an AUG initiation codon at about +55 with reference to Fig. 6 and extending for at least 25 amino acid residues without a stop codon. The precise ends of the leader sequences can be determined exactly only by reference to a cDNA clone, which is presently available only for *DCα* and *DCβ* or through homology to other cDNA clones in the case of *DXα* or *DXβ*. Thus all of the data obtained to date are compatible with the conclusion that *DXα* and *DXβ* are intact genes.

Homozygosity of Priess Cell DNA at the DC and DX Subregions. The Priess cell line has been typed serologically to be DR4/4, DC4/4, and SB3/4 (20, 21). Heterozygosity at the SB subregion has been confirmed by Southern blots (17) and by analysis of cosmid clones covering the SB subregion (7). Recently, Spielman *et al.* (15) reported that Priess cell DNA might be heterozygous in the DC subregion. Using a *DCα* DNA probe, they observed three *Eco*RI bands, 15.5, 12.5, and 5.0 kb, as well as three *Taq* I bands, 4.3, 2.6, and 2.1 kb on Southern blots, the first two in each case corresponding to different *DC(MB)α* alleles. However, genomic blots of Priess DNA prepared from our cell stock, as well as cells provided by J. G. Bodmer, showed that these two cell stocks are identical and do not yield the bands previously reported (15). In DNA from both cell stocks, two *Eco*RI bands, 6.3 and 5.1 kb, and two *Taq* I bands, 5.5 and 2.0 kb, hybridized with the *DCα* cDNA probe (Fig. 2*A*). The 6.3-kb *Eco*RI band and the 5.5-kb *Taq* I band correspond to the *DCα* gene, while the 5.1-kb *Eco*RI band and the 2.0-kb *Taq* I band correspond to the *DXα* gene. A genomic blot of *Hin*dIII-digested Priess DNA also showed two bands, one, 5.5 kb, encoding the *DCα* gene and the other, 2.6 kb, encoding *DXα* (4). In addition, genomic blots of DNA from both cell lines with the *DCβ* cDNA probe showed that they are identical and apparently homozygous for *DCβ* and *DXβ* (Fig. 2*B*). Therefore, no indication that the *DCα* subregion of Priess cell is heterozygous could be found. Moreover, the homozygosity at the DC subregion is supported by the fact that all of the eight cosmid clones independently isolated from the genomic library of Priess DNA have identical physical maps for many restriction enzymes (Fig. 1).

DISCUSSION

The number of *DCα*- and *DCβ*-related genes on human chromosome 6 has not been firmly established. Southern blot analyses using a *DCα* cDNA probe showed two α genes, named *DCα* and *DXα* (see also refs. 4, 13, and 14). Similar analyses using a *DCβ* probe have demonstrated two *DCβ*-related genes also (12). In this study, another *DCβ*-related gene, named *DXβ*, has also been detected (it has independently been found in two other laboratories †‡). These two β genes, *DCβ* and *DXβ*, are closely linked to the *DCα* and *DXα* genes, respectively. The gene organization of the *DCα–DCβ* and the *DXα–DXβ* pairs on cosmid clones have several common features, namely homologous nucleotide sequences, similar exon–intron structure, and the same orientation of the genes (tail-to-tail, i.e., 3′-to-3′). These similarities obviously suggest that the two pairs of α–β genes are derived from duplication of an ancestral gene pair. It is, therefore, also likely that the *DC* and *DX* gene pairs are neighboring in the MHC on chromosome 6. A hybridization study using human–mouse hybrid cells showed that the *DCα* and *DXα* genes are located on chromosome 6 (15), a conclusion that

†Larhammar, D., Gustafsson, K., Hyldig-Neilsen, J. J., Hammerling, U., Servenius, B., Rask, L. & Peterson, P. A., MHC Cloning Workshop, May 2–4, 1984, Strasbourg, France, abstr. p. 13.

‡Gorski, J., Rollini, P., Long, E. & Mach, B., MHC Cloning Workshop, May 2–4, 1984, Strasbourg, France, abstr. p. 18.

was also reached by studying deletion mutants (13). The distance between them is, however, not known.

Pairwise organization of an α and a β gene is a common feature of human and mouse MHC class II genes. In mouse, *I-E*α and β genes as well as *I-A*α and β genes are arranged in a tail-to-tail (3′-to-3′) manner (32). In man the SB subregion is composed of two pairs of neighboring genes, *SB*α–*SB*β and *SX*α–*SX*β (6, 7). These pairs are anomalously in head-to-head (5′-to-5′) orientation.

It has been recently shown that the upstream sequences of the class II genes have two homologous sequences (−69 to −78 and −98 to −110) (25, 26). A comparison of the *DC/DX* α and β genes as well as all the presently available upstream sequences firmly defines these two elements as well as additional α-gene- or β-gene-specific sequences that surround the −69 to −78 element. It is interesting to note that the central sequence of this element (A-T-T-G-G) is the reverse complement of a C-C-A-A-T box, but the significance of this region is not known. However, it seems likely, in view of recent studies showing that class II genes can be coordinately induced by γ-interferon (33), that these sequences may play a role in the coordinate expression of these genes either in normal development or in γ-interferon induction.

The *DC*α and *DC*β genes encode α and β subunits that form class II antigens immunologically recognized as DC (MB) determinants. The protein molecule that bears the MB3 supertypic specificity was isolated from Priess cells (DC4/4) by immunoprecipitation with a monoclonal antibody IVD-12, and a partial NH_2-terminal amino acid sequence was determined (34). The positions of several amino acid residues found in the α and β subunits are consistent with nucleotide sequences of the *DC*α (10) and *DC4*β (11) genes. It is not known whether *DX*α and *DX*β genes are expressed genes and to which family of serologically defined antigens they may correspond. However, since nucleotide sequences of the entire *DX*α and *DX*β genes show no indication that either is a pseudogene, it is quite possible, but still unproven, that the *DX*α and *DX*β genes encode DC-related molecules found in some B cell lines (35) or the recently isolated set of class II antigens that are closely related to, but different from, MB determinants (36). However, it should be kept in mind that, although both *DC*α and *DC*β are polymorphic genes, *DX*α and *DX*β are quite conserved, at least as evidenced by Southern blots of genomic DNA.

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