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Immunology

Cloning and sequence analysis of the human major histocompatibility complex gene *DC-3β*

(class II antigen/Southern blot analysis)

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ABSTRACT **The DC antigen is one of the class II major histocompatibility antigens involved in the regulation of the immune response. This molecule is a heterodimer composed of an α and a β chain. Southern blot analysis of several homozygous cell lines shows that there are two *DCβ* genes. The *DC-3β* gene, corresponding to a polymorphic restriction fragment, was cloned and sequenced and found to exist in five exons spanning 8 kilobase pairs of DNA. These exons correspond to the functional domains of the DCβ protein. Comparison of the β1 domains of known DCβ chains shows that the polymorphism is clustered in four regions. A similar comparison of the mouse Aβ sequences shows only two prominent diversity regions. The DCβ chain sequences are eight amino acids shorter than the Aβ chain sequences due to the elimination of a small exon by an aberrant splice acceptor.**

The class II antigens are encoded on chromosome 6 in the *HLA-D* region of the major histocompatibility complex (MHC) in man and on chromosome 17 in the *Ia* region of the mouse. These antigens are normally expressed on the surfaces of B cells, some human T cells, and macrophages (reviewed in refs. 1 and 2). They are glycosylated heterodimers consisting of an α (heavy) and a β (light) chain of approximately 33,000 and 28,000 daltons, respectively (3). The second extracellular domain of each of these chains (α2 and β2) shows significant homology to the α3 domain of class I MHC antigens, to β_2-microglobulin, and to the constant region of immunoglobulin molecules (1, 3). Although the details of the function of these antigens have yet to be elucidated, they play an integral role in regulation of the immune response and in communication between lymphocytes.

The number of genes encoding class II antigens in man is unclear; however, three regions encoding the DR, DC, and SB α and β chain genes have been studied. The mouse homologues for the DR and DC antigens are I-E and I-A, respectively. cDNAs (reviewed in refs. 1 and 2) and some genomic clones (4–8, 33) corresponding to α and β chain genes for each of these families have been isolated and sequenced. The compilation of the DNA sequences of these genes has contributed significantly to the understanding of the primary structure of the proteins they encode and has suggested possible mechanisms for the genesis of the polymorphism seen between alleles of these genes.

To determine the structure of the class II β genes and to compare DC allotypes, we have cloned and sequenced a *DC-3β* gene. The primary structure of this gene and the mapping of the transcription initiation site are discussed here. In addition, we compare the sequences of this gene to those of other *DCβ* genes and the homologous *I-Aβ* genes.

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MATERIALS AND METHODS

Cloning and Sequencing. A partial *Mbo* I genomic library was constructed in the EMBL3B vector (9) from the lymphoblastoid cell line WT49 (DR3,3), a homozygous line derived from an offspring of a consanguineous marriage. The library was amplified and then screened with the protein-encoding sequences (*Pst* I/*Eco*RI fragment) of a DCβ cDNA clone pIIβ1 (formerly pDRβ1) (10). Hybridization and washing of the filters were carried out as previously described (9). Subclones and deletions (11) were constructed from λ-42 DNA by using the pUC9, pUC12 (12), or M13 mp8 (13) cloning vectors to facilitate sequencing. Restriction fragments were sequenced according to the procedures described by Maxam and Gilbert (14) and phage M13 clones essentially according to Sanger *et al.* (15).

RNA Purification and Primer Extensions. Total RNA was prepared by using the guanidinium thiocyanate/cesium chloride procedure (9), and poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose chromatography (16). Primer extension reactions were carried out essentially as previously described, using a 5′-end-labeled synthetic oligonucleotide as a primer (17). This oligonucleotide, d(G-G-A-T-C-C-G-C-A-A-A-G-C-C-T-T-T-T-T-C-C), encodes the reverse complement of seven amino acids (−23 to −30) in the signal sequence.

RESULTS AND DISCUSSION

Cloning, Identification, and Sequencing. A genomic library from the homozygous lymphoblastoid cell line WT49 (DR3,3) was screened with a DCβ cDNA clone pIIβ1 (formerly pDRβ1) (10). Several clones were strongly positive. One of the clones, λ-42, was chosen for further analysis and a detailed restriction map was prepared (Fig. 1). The entire gene was sequenced (Fig. 2) in both directions or through overlapping clones (Fig. 1).

The comparison of the sequence of λ-42 (Figs. 2 and 3*A*) to the NH_2-terminal protein sequence of a DC-1β chain (18) (Fig. 3*F*) shows exact identity, indicating that λ-42 is a DCβ clone. Since WT49 is homozygous at the *DR* locus (DR3,3) and *DR3* is in linkage disequilibrium with DC-3, it is likely that λ-42 contains a *DC-3β* gene. However, at least two DC-like β chain genes may occur in the human genome (see below), and thus it is not possible to state unequivocally that the present gene encodes the serologically defined DC-3β specificity. The appropriate expression of this gene and assay of its serological reactivity will ultimately define its specificity. Comparison of the amino acid sequence to that of the cDNA clone pIIβ1 [isolated from Raji cells (DR3,6), Fig. 3*B*] (10) shows only one amino acid change, indicating that these two clones are likely to give rise to proteins with

Abbreviations: MHC, major histocompatibility complex; kb, kilobase pair(s); bp, base pair(s), TM, transmembrane.

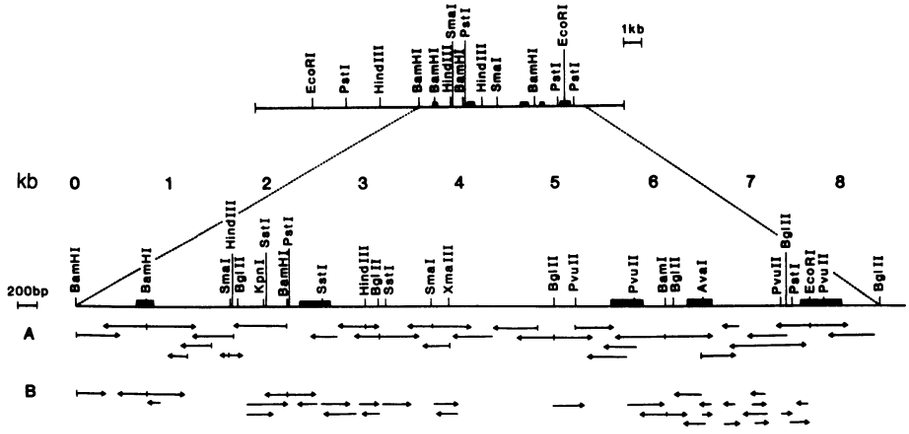

FIG. 1. Restriction map and sequencing strategy. A restriction map of clone λ-42 and a detailed map of the sequenced region are shown. Darkened boxes represent the exons of the *DC-3β* gene. (*A*) Direction of sequencing using the chemical degradation method (14). (*B*) Direction in which the M13 mp8 subclones were sequenced by using the dideoxy method (13, 15). kb, Kilobase pairs; bp, base pairs.

the same serological reactions—i.e., pIIβ1 most likely was derived from the *DR3*-containing haplotype of Raji cells.

Southern Blot Analysis of the DCβ Chain Gene in WT49 DNA. Southern blots using DNA from homozygous individuals and a variety of restriction enzymes have shown two to four prominent bands when probed with a *DCβ* cDNA, indicating the occurrence of several related genes (21, 22). One of the prominent bands was nonpolymorphic, while at least one other was polymorphic among the different cell lines. A similar analysis of the *DCβ* genes in the WT49 (DR3,3) cell line was carried out by Southern blotting (Fig. 4).

DNAs from three homozygous cell lines, WT49 (DR3,3), Priess (DR4,4) and LB (DR6,6) were digested with *Eco*RI. The *Pst* I/*Eco*RI fragment of pIIβ1 hybridizes to a prominent 13-kb polymorphic *Eco*RI fragment in the WT49 genome, which corresponds to the *DCβ* gene analyzed here. This is the only *Eco*RI fragment in this gene that will hybridize to the probe. The 18-kb band in Priess also corresponds to a single *DCβ* gene (found in cosmid T28L; K. Okada, personal communication) and probably to the gene cloned and sequenced by Larhammer *et al.* (8). The corresponding gene in LB DNA appears to have *at least* one additional *Eco*RI site within the corresponding *DCβ* gene, producing a prominent 2.4-kb band and two less prominent bands at 11 and 10 kb (or, alternatively, LB cells contain more *DCβ* genes than WT49 or Priess). These fragments are obviously those of a polymorphic gene. A prominent 6.5-kb band is nonpolymorphic with this enzyme within these three cell lines. In addition, several less strongly hybridizing nonpolymorphic bands are evident, some of which are washed away under higher stringency conditions. These bands may result from cross-hybridization to more distantly related *DCβ*-like genes, to pseudogenes, or to some other class II MHC genes, since the probe used contains the entire β2 domain that is conserved among class II genes. From these results we conclude that there are two major *DCβ*-like genes in the WT49 cell line, and the gene sequenced here is derived from a polymorphic locus.

Exon and Intron Organization of the *DC-3β* Gene. The *DC-3β* gene is divided into five exons. The first exon contains the 5′ leader sequence of the mRNA, a signal sequence, and the first four residues of the β1 domain of the mature protein. The second and third exons encode the two extracellular domains β1 (amino acid residues 5–94) and β2 (amino acid residues 95–188), respectively. The fourth exon encodes residues 189–228, including the connecting peptide, the transmembrane domain, and the first six residues of the cytoplasmic domain. The fifth and final exon encodes four residues of the cytoplasmic domain and the 3′ untranslated sequences. Exon boundaries were determined by comparison to the cDNA clone pIIβ1. All splice sites are in concordance with the consensus sequences for splice donors and acceptors (24). The arrangement of exons and introns in the *DCβ* gene is similar to that in other MHC and immunoglobulin genes in that the respective protein encoding domains are typically defined by their own exons.

(*i*) *Signal sequence.* The signal sequence is 32 amino acids long and 56% of these residues are hydrophobic, consistent with the composition of most signal sequences. Comparison of this signal sequence to other DCβs reveals some intraspecies homology among allotypes but little homology when compared to the mouse homologue, $IA^b\beta$ (Fig. 3).

(*ii*) *β1 domain.* The β1 domain contains two cysteine residues, which may form an intrachain disulfide bridge, and a glycosylation sequence Asn-Gly-Thr. Both of these structural features are typical of class II light chains. It has previously been demonstrated that the polymorphic regions of the class II molecules reside in the β1 domain (25). Comparison of the DC-3 β1 residues to those of a probable DC-4 allele (cosII-102, isolated from an individual homozygous for DR4) (8), a second cDNA clone pIIβ2 (isolated from Raji; DR3,6) (5) and a protein-derived sequence from a chemically isolated DCβ chain (18) (termed DCAA in this paper) show 15, 22, and 24 amino acid differences, respectively (Fig. 3), which are clustered in four regions (residues 28–32, 46–57, 66–77, and 84–90, boxed in Fig. 3).

The Aβ chains from the *d,k*, *b* and bm^{12} (5, 20, 31, 32) haplotypes are also compared to DC-3β in Fig. 3. These clustered amino acid changes are much less prominent in the mouse Aβ sequences and there appear to be fewer of them—i.e., less divergence appears to have occurred in the murine system. The NH_2-terminal sequences in the DC light chains are nearly identical for the first 24 residues of β1, while the corresponding residues in the mouse, notably residues 9–14, are very variable. A polymorphism also occurs in I-Aβ at residues 62–78, also seen in DCβ. Moreover, the functional significance of changes in this region has been clearly demonstrated by the occurrence of amino acid substitutions in the functionally altered $A\beta^{bm12}$ mutant at residues 67, 70, and 71, resulting from a gene conversion-like event with the *Eβ* gene as the donor (31, 32).

The DCα chain genes also display an allele-specific polymorphism with a similarly placed cluster at residues 45–56, and what appears to be a *DCα/DXα*-locus specific divergence at residues 63–70 (26). It is interesting to note that in the cluster at residues 84–90 (Fig. 3) there are no differences between DC-3β and the DC-4β sequence, while six out of eight differences are found when comparing the DC-3β sequence to the pIIβ2 and DCβAA sequences. Since there is more than one *DCβ* gene, this cluster (residues 84–90) may be analogous to the locus-specific cluster seen when comparing the *DCα* and the *DXα* genes. Alternatively, this region (residues 84–90) may be an additional polymorphic region among certain alleles.

(*iii*) *β2, transmembrane, and cytoplasmic domains.* The β2, transmembrane, and cytoplasmic domains are more

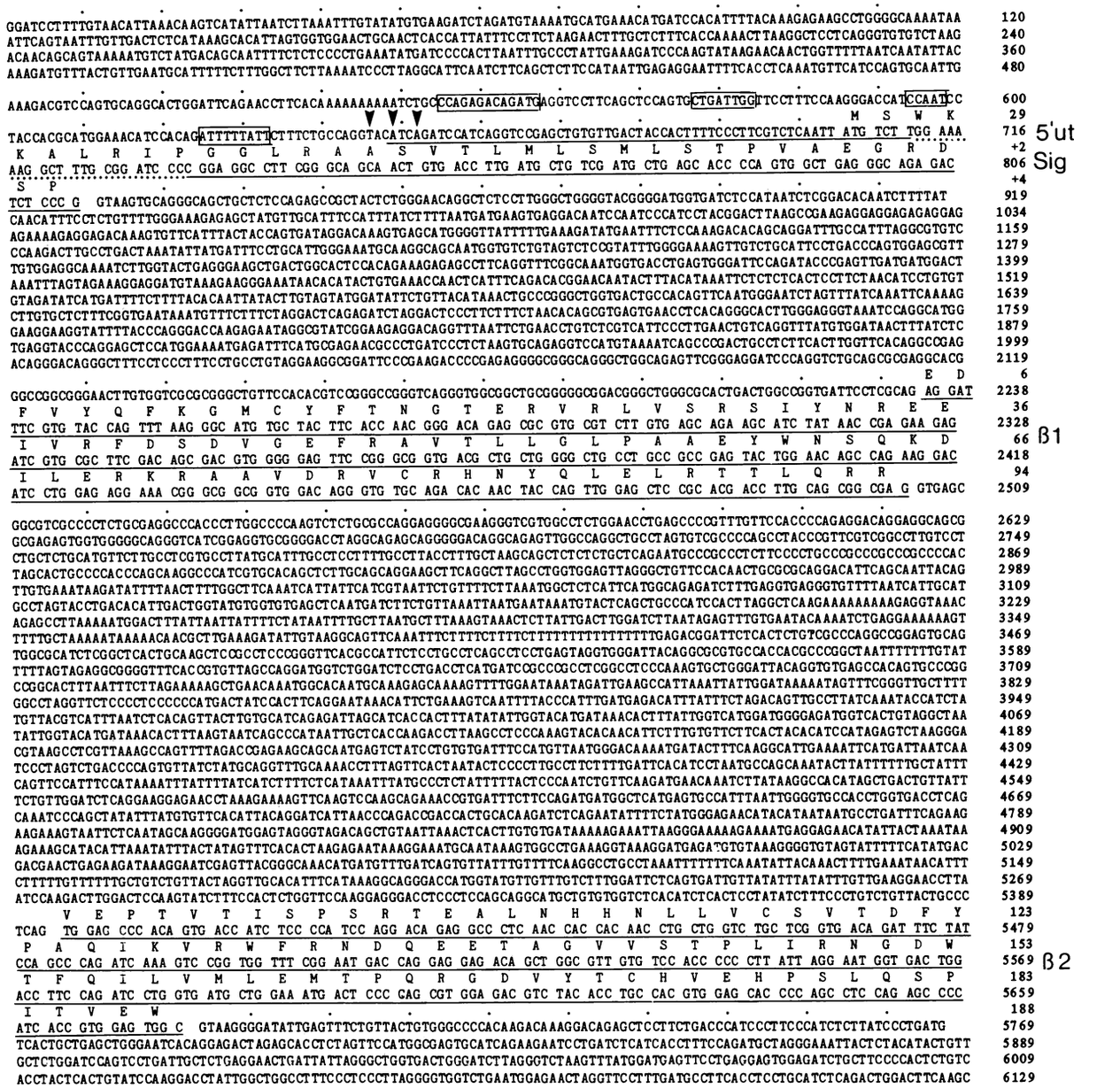

FIG. 2. Sequence of the *DC-3β* gene. The nucleotide sequence of *DC-3β* is shown, with the translated amino acids (standard one-letter code) above their respective codons. Underlined regions represent exons and dotted underlining at nucleotides 711–738 and 6786–6808 refer to the reverse complement of the oligonucleotide primer used in the primer extension experiments and the missing exon discussed in the text, respectively. Boxed regions 534–547 and 567–574 highlight sequences that are common to the upstream regions in all class II genes. Boxed regions for nucleotides 593–597, 625–633, and 7765–7770 highlight the "CAT," "TATA," and "ATTAAA" sequences, respectively. The arrows point to the initiation of transcription sites as determined from primer extension experiments. Sig, signal sequence; ut, untranslated sequences; TM, transmembrane sequence; CY, intracytoplasmic sequence.

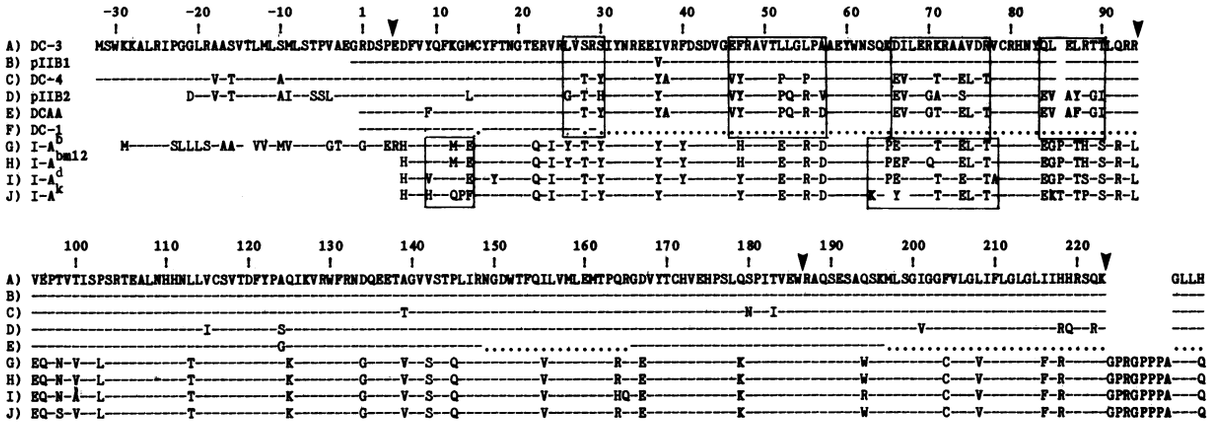

FIG. 3. Comparison of DCβ-like chains. The DC-3β amino acid sequence (A) is shown for comparison with B, pIIβ1 (10); C, DC-4β from cosII-102 (8); D, pIIβ2 (5); E, DCAA (18); F, DC-1β (19); G, $A^b\beta$ (5, 20); H, $A^{bm12}\beta$ (31); I, $A^d\beta$ (20); and J, $A^k\beta$ (20). Dashes indicate identities; dots represent undetermined residues; and gaps represent deletions in the amino acid sequence placed so as to maximize the homology between the proteins. Arrowheads show the intron–exon boundaries for the *DC-3β* gene. Boxes circumscribe clusters of amino acid polymorphisms.

highly conserved between the allotypes than the β1 domains. The β2 domain is homologous to the α2 domain of class II heavy chains, α3 domain of the class I MHC antigens, β_2-microglobulin, and the constant region domains of immunoglobulins (1, 2). Comparison of the DC-3β chain with other DCβ and Aβ light chains for the most part indicates that the amino acid changes in these regions are conservative where charged amino acids are involved (Fig. 3).

The DCβ cytoplasmic domain is also eight amino acids shorter than the domains of Aβ, DRβ, and Eβ. As noted in earlier reports (5, 7, 8) this could be due to the presence of an aberrant splice site eliminating an exon in the *DCβ* genes. The intron between the transmembrane exon and the 3′ untranslated exon was translated in all three reading frames. A sequence homologous to the exon in the Aβ genes was found in this intron in the *DC-3β* gene (indicated by a dotted underline in Fig. 2) and can be compared with the translated Aβ sequence and a similar sequence in DC-4β: I-$A\beta^b$, G-P-R-G-P-P-P-A; DC-3β, [G-P-E-G-P-P-A]; and DC-4β, [G-P-E-G-P-P-P-A] (brackets indicate an untranslated sequence). There are two anomalies in the nucleotide sequence of *DC-3β* in this region. First, the splice acceptor usually found as Y_n-N-Y-*A*-*G*/G (24) is Y_n-G-T-*A*-*A*/G. An *A*-*A* has not been found to serve as a splice acceptor site (24). Second, there are only seven instead of eight amino acids encoded before the splice donor is encountered. The sequence generated for the *DC-4β* gene (cosII-102) (8) shows a similar pseudo-exon containing the same aberrant splice acceptor, but this exon contains all eight amino acids. Because both alleles have the same aberrant acceptor and the DC-4β sequence has eight amino acids it seems likely that the loss of the proline codon in the DC-3β sequence occurred later in evolution than the loss of the splice acceptor.

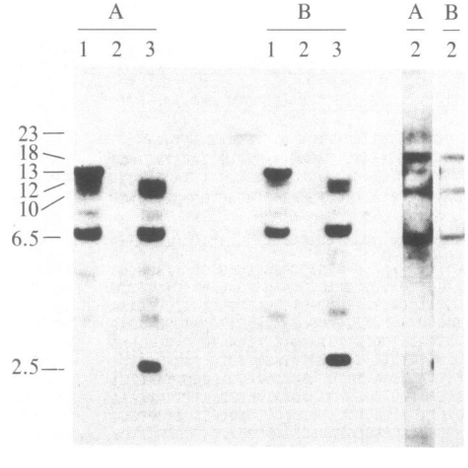

FIG. 4. Autoradiographs of Southern blots. Genomic DNA from three homozygous cell lines WT49 (DR3,3) (lanes 1), Preiss (DR4,4) (lanes 2), and LB (DRw6,6) (lanes 3) were digested with *Eco*RI. Each sample was split in half and electrophoresed on either side of the gel. Southern blotting was carried out as previously described (23). Hybridization was carried out with a nick-translated *Pst* I/*Eco*RI fragment of pIIβ1 (15) as previously described (9). The blot containing duplicate samples was washed in 300 mM NaCl/30 mM sodium citrate/0.5× Denhardt's solution/0.1% sodium dodecyl sulfate two times for 30 min at 68°C. The blot was cut in half. Blot A was further washed in 300 mM NaCl/30 mM sodium citrate and blot B in 30 mM NaCl/3 mM sodium citrate for 4 hr with four changes of their respective buffers at 68°C. A darker exposure of lane 2 for blot A and B is shown on the right. Positions of size markers are shown in kb on the left.

(iv) The 3′ untranslated domain. The 3′ untranslated portion of the *DC-3β* gene consists of approximately 350 nucleotides (underlined in Fig. 2). This sequence is extremely homologous to the 3′ ends of both the *DC-4β* and the pIIβ1 genes, differing at 8 and 2 nucleotides respectively. Since the cDNA clone pIIβ1 does not contain a poly(A) sequence we assume that it is just short of the poly(A) sequence and estimate the 3′ end of the mRNA to be about 20 nucleotides from the polyadenylylation signal, which in this case is A-T-T-A-A-A. A-T-T-A-A-A sequences have been found in several other genes (27), although A-A-T-A-A-A is more frequent. An A-T-T-A-A-A sequence is also present in both the *DC-4β* and pIIβ1 clones as well as in a DRβ cDNA clone. The sequencing of an additional 300 nucleotides past this point reveals no other polyadenylylation signal.

Nucleotide Polymorphism. A comparison of nucleotide differences between *DC-3β* and *DC-4β* or pIIβ2 shows that in β1 77% and 71% of the differences, respectively, are in the first and second positions of the codons (Table 1); in β2, 60% and 44%; and in TM, 0% and 37%. Moreover, for the most part the differences in β1 result in nonconservative amino acid replacements, whereas the β2 and TM domains show

Table 1. Nucleotide differences within codons

Domain compared with *DC-3β*	β1			β2			TM		
	1	2	3	1	2	3	1	2	3
DC-4β	4	11	6	1	2	2	0	0	0
pIIβ2	15	16	9	3	1	5	1	2	5

The numbers of nucleotide differences are tabulated for each position within the codon.

more third-position differences, and these nucleotide differences generally result in silent or conservative amino acid substitutions. A similar result was obtained when I-A^b was compared to I-A^d or I-A^k. Although the sample examined is small, this observation is consistent with that found for other MHC genes, such as *DCα* (26), *Aα* (28), and the variable region of immunoglobulin genes (29).

Gene conversion, unequal crossing-over events, and/or point mutations in functionally important areas followed by population selection are proposed as possible mechanisms by which clustered sequence polymorphisms can be generated (29). The former two are capable of causing rapid change between members of a gene family. A gene conversion-like event has been shown to be the cause of both the class I mutant, *bm1*, and the class II mutant, *bm12*, in the mouse (30–32). Whatever the mechanism, it is clear that the β1 domain is subject to different mutagenic and selection pressures than the other domains.

Mapping of the 5′ End of DC-3 β Chain mRNA. Primer extension experiments were carried out to determine the position of the *DC-3β* gene transcription initiation site. Reactions were carried out using poly(A)$^+$ RNA and total RNA from WT49 cells and 721.82 cells (Fig. 5). The 721.82 cell line, used as a control, contains a double haplotype deletion of the *DR* and *DC* loci (21). The primer extension reactions show three bands (arrows in Figs. 2 and 5) occurring at 58, 55, and 50 nucleotides 5′ to the ATG codon in the WT49 RNA track. No bands are seen in the 721.82 RNA track, which in this case serves as a control for artifacts. It is not uncommon to see several bands in primer extension experiments. These bands may represent multiple transcription initiation sites, regions of secondary structure that reverse transcriptase has trouble reading through, or transcription from another DC-like β gene (Fig. 4).

The Promoter. The DNA sequences 5′ to the transcription initiation site contain the standard signals for transcription. A TATA-like region, A-T-T-T-T-T-A-T-T; and a CAT box, C-C-A-A-T, are found 20 and 51 bp 5′ to the start of the transcription. In addition, the *DC-3β* gene also has additional sequences C-C-A-G-A-G-A-C-A-G-A-T-G and C-T-G-A-T-T-G-G located 121 and 78 bp 5′ to the start of the transcription (boxed sequences in Fig. 2). These sequences, as noted previously (6, 33), are common to *Eα*, *Eβ*, and *DRα*. In addition these sequences are found in both the DC*α* and DX*α* sequences (J. Lillie, personal communication). It may be that these sequences are responsible for the specific regulation of these genes during the development of an immune response.

The *DC-3β* gene presented in this paper has all the features characteristic of a functional gene. This statement can be verified only by transfecting this gene into cells and assaying for its expression.

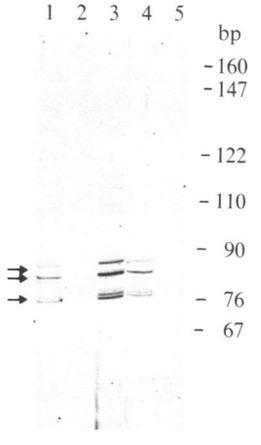

FIG. 5. Mapping the 5′ end of the DC-3β mRNA. Autoradiograph of primer extension experiment reactions carried out on poly(A)$^+$ RNA from an equivalent of 20 μg of total RNA from WT49 (lanes 1 and 3) and 721.82 cells (lane 2) or 20 μg of total RNA from WT49 (lane 4) and 721.82 (lane 5). The arrows point to the major bands representing the 5′ ends of the DC-3β mRNA.

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