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Alternative splicing of HLA-DQB transcripts and secretion of HLA-DQ β-chain proteins: Allelic polymorphism in splicing and polyadenylylation sites

(major histocompatibility complex/class II antigens/RNA processing)

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ABSTRACT **HLA class II antigens are highly polymorphic cell-surface proteins involved in initiation and regulation of the immune response. Allelic sequence variation primarily affects the structure of the first external domains of α and β component chains. Here we provide evidence for other types of allelic polymorphism for the genes encoding these chains. Sequences of two cDNA clones corresponding to HLA-DQB mRNAs from an *HLA*-homozygous cell line exhibit both alternative splicing and read-through of polyadenylylation. Furthermore, alternative splicing that deletes the transmembrane exon is associated with only a subset of *HLA-DQB* alleles, while the polyadenylylation-site read-through is found in a larger subset. This suggests that polymorphic cis-acting elements within the *HLA-DQB* gene control both processing steps. Proteins, presumably encoded by alternatively spliced mRNAs lacking transmembrane exons, are immunoprecipitated with a monomorphic monoclonal antibody directed against HLA-DQ. These proteins are found in supernatants of cultured cell lines for which secretion is predicted, but not in those of cell lines that do not contain alternatively spliced mRNAs.**

HLA class II antigens are heterodimeric cell-surface antigens present primarily on B cells and macrophages (1–3). Biochemical and molecular analysis of class II proteins indicated that α and β chains associate soon after synthesis within the endoplasmic reticulum, and both chains span the membrane (4). The best-characterized function of major histocompatibility complex class II molecules of both man and mouse is their role as restriction elements for presentation of foreign antigen to helper T cells. Foreign antigens are thought to be internalized and processed by proteolytic digestion and then returned to the cell surface in association with recycling class II antigens (3).

Polymorphic residues in N-terminal domains of class II α and β chains influence interactions of the molecules with peptide antigens and T-cell receptors and are the basis for HLA restriction of effector–target cell interactions. But polymorphism other than that affecting the structure of external domains is also observed. Comparisons of sequences of different HLA-DQα (hereafter referred to as DQA) cDNAs showed alternative splicing dependent on sequence polymorphisms of splice acceptor sites (5, 6). However, splicing differences do not involve coding regions and, thus, should not affect the structure of the protein. In addition, alternative splicing can create HLA-DP, -DQ, and -DR (hereafter referred to as DP, DQ, and DR) β chains with different cytoplasmic residues beyond the transmembrane sequence (7–9).

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We undertook sequence analysis of HLA class II cDNAs from *HLA*-homozygous cell lines to define polymorphisms within allelic groups more precisely. To our surprise, one DQB cDNA clone lacked an exon coding for a transmembrane (TM) region (DQBΔTM). In addition to the alternative splice junction, the clone includes 417 nucleotides downstream from the normal polyadenylylation site. Furthermore, we detected truncated DQ β chains in culture supernatants from cell lines that produce alternatively spliced DQBΔTM mRNAs.

MATERIALS AND METHODS

Cell Lines. *HLA*-homozygous, consanguineous Epstein–Barr virus-transformed B-cell lines were obtained from S. Y. Yang and B. Dupont (Sloan–Kettering, New York). All cell lines were maintained in RPMI 1640 medium supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (Whittacker M.A. Bioproducts), 2 mM glutamine, and 100 μg of penicillin and 100 μg of streptomycin per ml (all from GIBCO).

cDNA Clones. RNA was extracted from cell line DBB (workshop no. 9052) by the method of Favaloro *et al.* (10). Poly(A)$^+$ RNA was selected, and a library was generated as described by Sartoris *et al.* (11). Inserts were subcloned into phage M13, and sequencing was performed by using the chain-termination method (12) with Sequenase from United States Biochemical with universal primers and class II β chain-specific primers (13).

Analysis of RNA. Cytoplasmic Poly(A)$^+$ RNA (1 μg) was subjected to electrophoresis in 1% agarose/formaldehyde gels as described (14) and transferred to Zetabind filters in 20× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7).

DNA Fragments and Probes. DNA fragments were isolated from the DQBΔTM cDNA clone by digestion with *Pst* I and *Pvu* II. An 800-nucleotide *Pst* I–*Pvu* II fragment (designated CR) corresponding to the complete coding region and a small part of the 3′ untranslated region and a 400-nucleotide *Pst* I–*Pvu* II fragment (designated 3′FL) including only 3′ flanking-region sequences plus about 50 nucleotides from the plasmid were isolated by agarose gel electrophoresis and binding and elution from NA45 membranes (Schleicher & Schuell). Fragments were labeled by random priming with hexanucleotide fragments (15). Hybridization of Zetabind filters was conducted as recommended by AMF-CUNO (technical bulletin). Probes were used at concentrations of 2×10^6 cpm/ml of hybridization solution. Stringency of the final washes was 0.1× SSC/1% sodium dodecyl sulfate at 60°C.

Abbreviations: TM, transmembrane; mAb, monoclonal antibody.

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Oligonucleotide Probes. Oligonucleotide probes designated DQ+TMβ and DQΔTMβ were labeled with [γ-^{32}P]ATP to an activity of 1–2 $\times$ 10^9 cpm/μg with phage T4 polynucleotide kinase (Boehringer Mannheim; 3′ exonuclease-free). Temperatures for hybridizations were calculated with the formula: [4(G + C) + 2(A + T)] $\times$ 20/no. of nucleotides in the probe (16), and hybridizations were carried out as described (11).

Analysis of Secreted Proteins. A total of 4 $\times$ 10^6 B lymphoblastoid cells was incubated with 1.5 mCi (1 Ci = 37 GBq) of [^{3}H]leucine (Amersham) in 1 ml of leucine-free RPMI 1640 medium supplemented with 10% fetal bovine serum and L-glutamine for 18 hr at 37°C in 24-well plates. At harvest, the supernatant was saved, and cells were lysed with 1 ml of 1% octaethylene glycol dodecyl ether (C12E8; Nikkol, Tokyo) in 0.05 M Tris·HCl/0.15 M NaCl, pH 8.0, containing 0.05 mM phenylmethylsulfonyl fluoride and 0.1 mM N^α-(*p*-tosyl)lysine chloromethyl ketone for 30 min on ice. The lysate was centrifuged at 15,000 $\times$ *g* for 10 min at 4°C and then stored at −80°C.

For biochemical analysis of secreted proteins, supernatants and lysates were precleared by three rounds of incubation with 200 μl of a 50% suspension of protein A-Sepharose 4B. For specific immunoprecipitations, 50 μl of a 50% suspension of monoclonal antibodies (mAbs) covalently coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala) were incubated overnight at 4°C with either labeled lysates or supernatants (5 times the volume of supernatant was used per volume of lysate). mAbs used were: anti-DQ, designated 20.4D7.2 [or SFR20-DQM (17)]; anti-HLA class I, designated W6/32 (18); and anti-IgM, designated 2C3 (19). Eluted antigen was electrophoresed under reducing conditions in 10.5% sodium dodecyl sulfate/PAGE. Gels were prepared for fluorography with Amplify (DuPont) and then exposed to Kodak X-AR5 film.

RESULTS

DNA Sequence of the DQBΔTM cDNA Clone. The sequence for our unusual cDNA clone and the corresponding amino acids of a putative protein are shown in Fig. 1. Comparison

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                                             -32
                                             Met Ser Trp Lys Lys Ala
GGTGAGCTCGGTTGACTACCACTACTTTTCCCTTCGTCTCAATT ATG TCT TGG AAG AAG GCT          62

                        -20                                         -10
Leu Arg Ile Pro Gly Gly Leu Arg Val Ala Thr Val Thr Leu Met Leu Ala Met
TTG CGG ATC CCT GGA GGC CTT CGG GTA GCA ACT GTG ACC TTG ATG CTG GCG ATG     116

                            -1  +1                                  10
Leu Ser Thr Pro Val Ala Glu Gly Arg Asp Ser Pro Glu Asp Phe Val Tyr Gln
CTG AGC ACC CCG GTG GCT GAG GGC AGA GAC TCT CCC GAG GAT TTC GTG TAC CAG     170
                                                 Δ
                                        20
Phe Lys Gly Met Cys Tyr Phe Thr Asn Gly Thr Glu Arg Val Arg Leu Val Thr
TTT AAG GGC ATG TGC TAC TTC ACC AAC GGG ACG GAG CGC GTG CGT CTT GTG ACC     224

    30                                      40
Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr Ala Arg Phe Asp Ser Asp Val Gly Val
AGA TAC ATC TAT AAC CGA GAG GAG TAC GCA CGC TTC GAC AGC GAC GTG GGG GTG     278

            50                                      60
Tyr Arg Ala Val Thr Pro Leu Gly Pro Pro Asp Ala Glu Tyr Trp Asn Ser Gln
TAT CGG GCG GTG ACG CCG CTG GGG CCG CCT GAC GCC GAG TAC TGG AAC AGC CAG     332

                    70                                      80
Lys Glu Val Leu Glu Arg Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn
AAG GAA GTC CTG GAG AGG ACC CGG GCG GAG TTG GAC ACG GTG TGC AGA CAC AAC     386

                            90                                      100
Tyr Gln Leu Glu Leu Arg Thr Thr Leu Gln Arg Arg Val Glu Pro Thr Val Thr
TAC CAG TTG GAG CTC CGC ACG ACC TTG CAG CGG CGA GTG GAG CCC ACA GTG ACC     440
                                                 Δ
                                        110
Ile Ser Pro Ser Arg Thr Glu Ala Leu Asn His His Asn Leu Leu Val Cys Ser
ATC TCC CCA TCC AGG ACA GAG GCC CTC AAC CAC CAC AAC CTG CTG GTC TGC TCA     494

    120                                     130
Val Thr Asp Phe Tyr Pro Ala Gln Ile Lys Val Arg Trp Phe Arg Asn Asp Gln
GTG ACA GAT TTC TAT CCA GCC CAG ATC AAA GTC CGG TGG TTT CGG AAT GAC CAG     548

            140                                     150
Glu Glu Thr Thr Gly Val Val Ser Thr Pro Leu Ile Arg Asn Gly Asp Trp Thr
GAG GAG ACA ACT GGC GTT GTG TCC ACC CCC CTT ATT AGG AAC GGT GAC TGG ACC     602

                    160                                     170
Phe Gln Ile Leu Val Met Leu Glu Met Thr Pro Gln Arg Gly Asp Val Tyr Thr
TTC CAG ATC CTG GTG ATG CTG GAA ATG ACT CCC CAG CGT GGA GAC GTC TAC ACC     656

                            180                                     190
Cys His Val Glu His Pro Ser Leu Gln Asn Pro Ile Ile Val Glu Trp Arg Leu
TGC CAC GTG GAG CAC CCC AGC CTC CAG AAC CCC ATC ATC GTG GAG TGG CGG CTC     710
                                                                 Δ
Leu His ***
CTG CAC TGA CTCCTGAGACTATTTTAACTGGGATTGGTTATCACTTTTCTGTAACGCCTGCTTGTCCCTG   780
CCCAGAATTCCCAGCTGCCTGTGTCAGCCTGTCCCCCTGAGATCAGAGTCCTACAGTGGCTGTCACGCAG     850
CCACCAGGTCATCTCCTTTCATCCCCACCTCAAGGCTGATGGCTGTGACCCTGCTTCCTGCACTGACCCA     920
GAGCCTCTGCCTGTGCACGGCCAGCTGCGTCTACTGAGGCCCCAAGGGGTTTCTGTTTCTATTCTCTCCT     990
CAGACTGCTCAAGAGAAGCACATGAAAACCATTACCTGACTTTAGAGCTTTTTTACATAATTAAACATGA    1060
TGAGTT|ATCTCCTGTATTCTCAACTTCCTTAATTGAGCAGAGGCAGGAAATCACTGCAGAATGAAGGAAC   1130
CCTTGAGGTATAGACCCAGCCAACCTGTGCCCAGAAGGAGGGTTGTACCTTGAAAAGACACTGAAAGAAT    1200
GGGTGCAAATTTGTCAGGGTGGGCAGAGGAGGTAGAAGATCAACTCAGTTGTCGCATCATTCATGGTTCT    1270
ATATTGATGTTCTTCAAGTCAGTGGCCTGAGATATCCCAGCCTCTCTTCTGGTTTGGTGAGTGCTATATA    1340
AAGCATGGTAGTGGAATTGTTTGGGGGCAGACATATGACCCTTGGTCACTGGTGTTTCAAACATTCTGGA    1410
CACATCAATAGTCAAGAATAATTTTTACTTTTAAGAAGCATAACCAGCAATAAAAGTATTATTTTTGAGG    1480
TCT
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FIG. 1. Sequence of the DQBΔTM cDNA clone. A DQB cDNA clone was isolated from a library produced from the *DR 7, DQw9* cell line DBB as described (11). Numbers above the amino acid sequence denote their position in the polypeptide chain, with +1 representing the N-terminal residue in the mature peptide. Numbers in the right margin indicate the position of the last nucleotide in the line. Open triangles denote exon boundaries; boxes include cysteine residues, a glycosylation site (Asn-Gly-Thr), and two polyadenylylation sites ATTAAA and AATAAA. The vertical line after nucleotide 1049 shows the site of termination of a shorter common DQB+TM cDNA clone isolated from the same library.

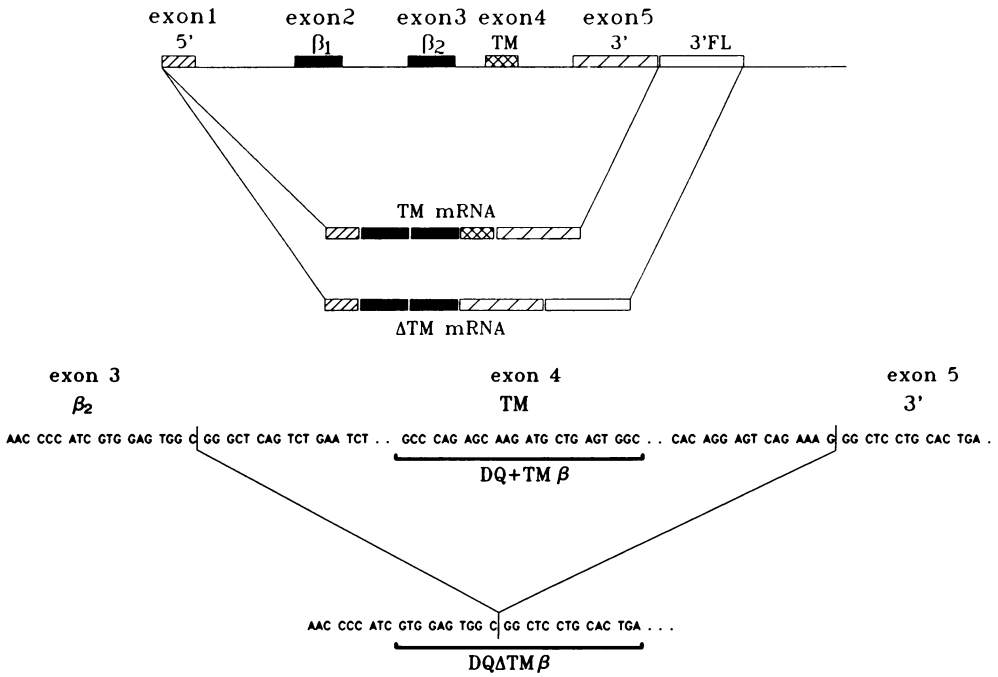

FIG. 2. (*Upper*) Schematic map of the *DQ* β-chain gene, *HLA-DQB*, and alternative splicing and polyadenylylation. The DQβ gene consists of five exons encoding the 5′ untranslated region and signal peptide (exon 1); the β_1 domain (exon 2); the β_2 domain (exon 3); the connecting peptide, TM region, and a part of the cytoplasmic tail (exon 4); and the remainder of cytoplasmic tail and the 3′ untranslated region (exon 5) (20, 21). The first RNA product represents the common form of DQ β-chain, which is also found in our library. The second RNA product represents the unusual DQΔTM β chain produced by alternative splicing and read through of polyadenylylation as found in the cDNA clone in Fig. 1. (*Lower*) Sequences of functions between exons 3 and 4, 4 and 5, and 3 and 5 in cDNA clones. Two oligonucleotides, DQ+TMβ and DQΔTMβ (underlined in the sequences), were synthesized in the antisense orientation. These oligonucleotides should recognize specifically the mRNA including and lacking the TM exon, respectively. Both sequences are highly conserved between *DQ* β-chain alleles, including *DQw2* (ref. 21 and J.S.L., unpublished data).

with genomic sequences for the *DQB* gene (human gene *HLA-DQB*) (20, 21) and for a common DQB+TM cDNA clone also found in our library showed that in our DQBΔTM cDNA, exon 3 was fused to exon 5 between nucleotides 705 and 706 in the codon for residue 189. Though exon 4 was not included, alternative splicing did not shift the reading frame for this clone, and a protein should terminate with the same four amino acids as found in the common DQ β chain. Moreover, the noncanonical polyadenylylation site found at nucleotide 1049 was not recognized as in other DQB cDNA clones, and the 3′ untranslated region was extended to the next polyadenylylation site (nucleotide 1459). Schematic maps of the *DQB* gene and two mRNA products represented by clones isolated from the DBB cDNA library are shown in Fig. 2 *Upper*. Because exon 4 is absent, the DQBΔTM RNA should encode a protein that cannot be anchored in the membrane via its own TM sequence.

Alternative Splicing of DQB RNAs in Different Haplotypes. The discovery of a cDNA clone that could correspond to a secreted DQ β chain or a β chain anchored to the membrane by a phospholipid linkage, as for the Thy-1 antigen (22), prompted us to look for similar RNAs in cells of different *DQ* specificities. Sequences corresponding to splice junctions for two DQB transcripts are shown in Fig. 2 *Lower*. We synthesized oligonucleotides that would hybridize specifically to each mRNA to determine whether similar mRNAs are produced in cells of other *DQ* specificities. These oligonucleotides are not highly polymorphic and, thus, should hybridize with equal efficiency to RNAs of different alleles. The DQ+TMβ oligonucleotide detected a major DQB mRNA in all cell lines and a minor mRNA in some (Fig. 3*A*). However, when the identical RNA (Northern) blot was hybridized with the DQΔTMβ oligonucleotide in Fig. 3*B*, we detected alternatively spliced mRNAs for only a subset of *DQB* alleles. Thus, lack of hybridization in lanes a, b, f, h, and i is not due to an absence of DQB mRNA in these samples. Lane j represents DBB cell RNA from which the cDNA library was made. Thus, the DQΔTMβ oligonucleotide is specific for alternatively spliced mRNAs.

As another control in these hybridizations, we used a probe (CR) containing the coding region and a small part of the 3′ untranslated region from the DQBΔTM clone. Fig. 3*C* shows that, as with the DQ+TMβ oligonucleotide probe, some RNA samples contained two DQB RNAs, while others contained only one. Finally, we used the 3′ flanking sequence

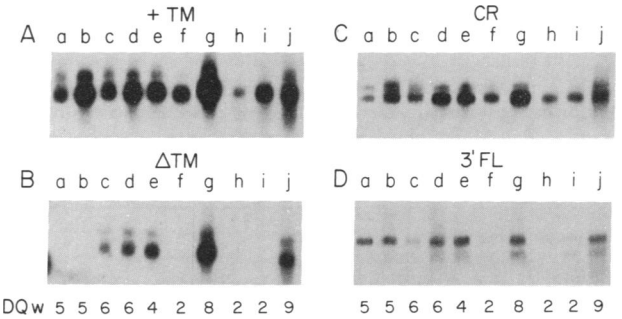

FIG. 3. Detection of DQB transcripts arising from several different alleles of *DQ*. A panel of cell lines established and studied in the Tenth International HLA Workshop were cultured, and polyadenylylated cytoplasmic RNA was isolated from several. These lines represented different groups based on *HLA-DR* typing. The same filter was probed in *A* and *B*, and another filter was probed for *C* and *D*. RNA samples in lanes were: a, MZ07082 (*DR 1*); b, KAS116 (*DR 1*); c, SCHU (*DR 2*); d, WT8 (*DR 2*); e, RSH [*DRw18* (*DR 3*)]; f, VAVY (*DR 3*); g, YAR (*DR 4*); h, BH (*DR 7*); i, PLH (*DR 7*); and j, DBB (*DR 7*). *DQw* types are designated below each sample in *B* and *D*. (*A*) Hybridization with DQ+TMβ. (*B*) Hybridization with DQΔTMβ. (*C*) Hybridization with the coding region (CR) probe. (*D*) Hybridization with the 3′ flanking sequence (3′FL) probe.

included in our novel DQB cDNA as a probe (3'FL; Fig. 3*D*). This too detected two mRNAs of different lengths in several cell lines; cells that produced alternatively spliced mRNAs also showed evidence for polyadenylylation read-through, but read-through occurred in the absence of alternative splicing in some samples (Fig. 3 *B* and *D*, lanes a, b, and i). Theoretically four mRNAs can be generated from the *DQ* β-chain gene. RNAs can include or exclude the TM exon and terminate at the first or second polyadenylylation site. Nevertheless, only inclusion or exclusion of exon 4 would be reflected in the structure of a possible protein.

Table 1 summarizes the results shown in Fig. 3. Although our survey was limited, DQBΔTM mRNA was conspicuously absent in B lymphoblastoid cells of *DQw5* specificities and also of *DQw2*. Levels of DQBΔTM RNA were approximately 10–20% of the levels of the common DQ+TM β-chain RNA (data not shown). Polyadenylylation read-through, in contrast to alternative splicing, occurred for *DQw5* alleles but was absent or greatly reduced in frequency for some *DQw2* alleles. Consequently, alternative splicing and read-through are not in complete association.

Analysis of DQ Products in Culture Supernatants. Our identification of DQBΔTM RNAs led us to search for a secreted DQ molecule in supernatants of cell lines. Four lines that we analyzed in our Northern blotting experiments were labeled for 16 hr with [^{3}H]leucine, and products were immunoprecipitated from cell lysates and supernatants. Two cell lines for which we predicted secreted DQ β-chains, YAR and RSH, and two lines which should not secrete these proteins, VAVY and MZ020782, were chosen. Fig. 4 shows that culture supernatants from YAR (Fig. 4*A*) and RSH (Fig. 4*D*) contain proteins that migrate behind the 29-kDa marker and precipitate specifically with the DQ monomorphic antibody 20.4D7.2 [also called SFR20-DQM (17)]. This antibody reacts with separated DQ β chains in immunoblots (S.F.R., unpublished data). In the experiments, DQ α and β chains are not readily detectable in lysates of YAR or MZ020782. Perhaps protein turnover during the long labeling interval lowers specific activity of the [^{3}H]leucine, so DQ chains (which are not as abundant as DR chains) are more difficult to detect. Nevertheless, lysates for VAVY and RSH appeared to contain proteins migrating just slower than the 29-kDa marker, consistent with the normal migration of DQ chains in these gels (24). In particular, the cell lysate for RSH appeared to contain both the DQ heterodimer and a molecule migrating ahead of the complex and also found in the supernatant. Calculated molecular weights for unglycosylated protein backbones of mature DQΔTM and DQ+TM β chains are 21 and 25 kDa, respectively. Thus, the mobility of the product precipitating with 20.4D7.2 from the YAR and RSH supernatants is consistent with deletion of 36 amino acids or about 4 kDa from the protein backbone by alternative splicing.

Table 1. Alternative splicing and polyadenylylation of HLA-DQB transcripts for different alleles

Cell line	Specificities*	Alternative splicing	Poly(A)$^+$ RNA read-through
MZ07092	*DR 1, DQw5(1.1)*	−	+
KAS116	*DR 1, DQw5(1.1)*	−	+
SCHU	*DR 2, DQw6(1.2)*	+	+
WT8	*DR 2, DQw6(1.2)*	+	+
RSH	*DRw18(3), DQw4(Wa)*	+	+
VAVY	*DR 3, DQw2*	−	−
YAR	*DR 4, DQw8(3.2)*	+	+
BH	*DR 7, DQw2*	−	−
PLH	*DR 7, DQw2*	−	+
DBB	*DR 7, DQw9(3.3)*	+	+

*According to proposed nomenclature from the Tenth International Histocompatibility Workshop, 1987 (23). Designations in parentheses correspond to previous specificities.

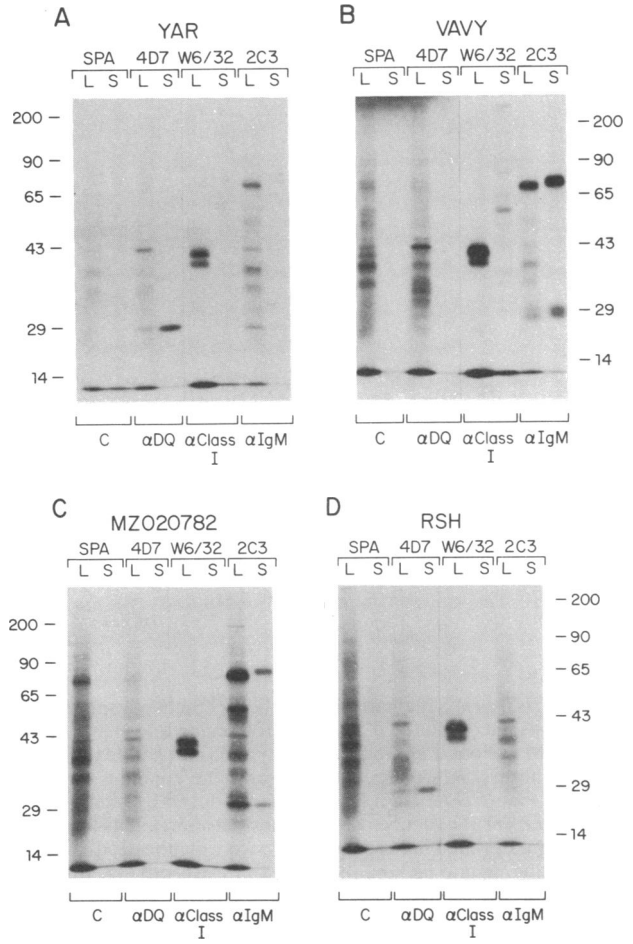

FIG. 4. Analysis of cell-associated and secreted protein products of *HLA*-homozygous cell lines. [^{3}H]Leucine-labeled proteins from cytoplasmic lysates (lanes L) and supernatants (lanes S) of four cell lines were analyzed by immunoprecipitation and sodium dodecyl sulfate/PAGE. SPA, *Staphylococcus* protein A-Sepharose; 4D7, anti-DQ (αDQ) mAb 20.4D7.2; W6/32, anti-class I (αclass I) mAb; and 2C3, anti-IgM (αIgM human) mAb. (*A*) YAR cell line. (*B*) VAVY cell line. (*C*) MZ020782 cell line. (*D*) RSH cell line.

No specific products were precipitated by 20.4D7.2, the DQ-specific antibody, from culture supernatants of MZ020782 and VAVY cell lines. Controls for these experiments were *Staphylococcus* protein A; the antibody W6/32, which recognizes HLA class I proteins (18); and the antibody 2C3, which recognizes human IgM (19). The DQ-specific band did not appear to result from fortuitous precipitation of a comigrating immunoglobulin light chain because the anti-IgM precipitates were strongest from the two cell lines (VAVY and MZ020782) that do not produce the secreted DQ-specific band. Thus, we conclude that YAR and RSH produce secreted DQ β chains that are the likely products of alternatively spliced mRNAs. We have not observed an alternatively spliced mRNA or a possible protein corresponding to a DQ α chain in our experiments, although these experiments do not exclude the possibility that both α and β chains are part of a secreted heterodimer.

DISCUSSION

Alternatively spliced DQBΔTM mRNAs are found in cytoplasmic RNA of several cell lines that we have analyzed. The unusual splicing that deletes the TM exon in these examples is distinct from that previously observed for DQB mRNA,

which showed alternative splicing affecting cytoplasmic and 3′ untranslated exons (8). All of the lines we studied generate a "normal" DQB+TM transcript as well. Association of exon 4 alternative splicing with particular alleles of *DQ* suggests that controlling elements for splicing patterns for DQB mRNAs are within the *DQB* gene or closely linked. Comparisons of genomic sequences for these different *DQB* alleles and construction of hybrid genes using genomic clones should help to identify sequence elements important in controlling the splicing apparatus.

Analysis also showed additional sequences at the 3′ end of a DQB cDNA clone. The first polyadenylylation signal, ATTAAA, located at nucleotide 1049 in the insert, was apparently read through, and the second signal at nucleotide 1459 was recognized (Fig. 1). It is intriguing that read-through of polyadenylylation occurs in all of the lines we tested, with the exception of those with the *DQB* allele *DQw2*, whereas alternative splicing occurs in all but *DQw2* and *DQw5* cells. Thus exon 4 alternative splicing and read-through are not in complete association.

At least one HLA class I allele, *A24*, produces an alternatively spliced mRNA missing the TM exon and a secreted protein bound to β_2-microglobulin (25). We have detected DQ-specific proteins in supernatants of cell lines that express DQBΔTM RNA (Fig. 4). Only one DQ-specific band was observed in immunoprecipitates of supernatants, and we conclude that because the antibody 20.4D7.2 recognizes the DQ β chain in immunoblotting experiments (S.F.R., unpublished data), the molecule corresponds to DQΔTM β chain. α and β chains of HLA class II antigens normally associate soon after their synthesis in the endoplasmic reticulum and appear at the plasma membrane as heterodimers (4). In the absence of its partner, a single class II chain is not usually transported to the cell surface (26). Thus, it is possible but seems intuitively unlikely that the DQΔTM β chain would be secreted as a single chain. We do not envision a DQ α-chain molecule linked to the membrane via its TM sequence bound with a "secreted" form of the DQ β peptide, because the hydrophobic TM regions of α and β chains probably interact within the membrane, and only one chain might not be stable (27). A more likely explanation for our inability to detect a candidate for a DQΔTM α chain in immunoprecipitates is that the α/β heterodimer may be less stable in the absence of its TM region, and the chains may separate before or during immunoprecipitation. In an effort to detect an α chain, two-dimensional gels were performed and suggest the possibility of a DQΔTM α-chain molecule (data not shown). Nevertheless, more rigorous studies are needed to identify the protein as an α chain. We have analyzed RNAs by Northern blotting with oligonucleotides from exon 3 to the 3′ untranslated region of DQA (two possible sequences were chosen) but did not detect equivalent molecules. Nevertheless, if an alternative splicing event joins the splice donor sequence adjacent to the 3′ end of exon 3 to a sequence downstream of the 3′ untranslated region exon, we would not have detected the RNA.

One of the most intriguing phenomena regarding *HLA* genes is their allelic association with diseases of autoimmune etiology (28). Explanations for these associations with class II molecules have dealt primarily with polymorphisms in coding regions of N-terminal exons (29). However, attempts to find correlations higher than those found with serological epitopes between allelic sequences and diseases have often failed. Secretion of products of some class II alleles could profoundly affect interactions between effector and target cells in an immune response and, thus, might influence disease susceptibility. For example, murine class II determinants have been found repeatedly on protein factors, however elusive, that are thought to function in suppression (30). Relative levels of class II antigen expression may also control myriad interactions between cells during an immune response. Recently, it was shown that ATTTA sequences in 3′ untranslated regions of some growth factors and oncogenes can affect stability of their corresponding mRNAs (31). Some *DQA* and *DQB* alleles contain these sequences (C. Hume and J.S.L., unpublished observations). It is feasible that such polymorphic controlling elements or those as yet unidentified could play a role in regulating expression of different *HLA* alleles. We suggest that certain associations of *HLA* class II alleles and diseases may be explained by cis-linked control of alternative splicing and quantitative regulation of class II mRNA levels.

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