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Unusual RNA splicing generates a secreted form of HLA-A2 in a mutagenized B lymphoblastoid cell line

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Human class I major histocompatibility antigens (HLA-A, -B and -C) are integral membrane glycoprotein heterodimers. A mutagenized B lymphoblastoid cell line has been previously shown to synthesize two forms of the HLA-A2 antigen; a minor form which remains cell-associated at all times, and an abundant form, which is secreted. The present study reports the isolation of cDNA clones for both the wild-type HLA-A2 molecule synthesized by the parent cell line and the secreted molecule synthesized by the mutant cell line. A comparison of their structures indicates that transcripts encoding the mutant HLA-A2 molecule lack the 117 nucleotides encoded by exon five of the HLA-A2 gene. This exon encodes the hydrophobic amino acids which are thought to anchor the polypeptide in the plasma membrane. This result supports an alternative splicing model to explain the phenotype of the mutant cell line. Further, it implies that information encoded in exon five is essential for anchoring class I antigens in the plasma membrane. The potential for a similar splicing mechanism to generate soluble forms of class I antigens *in vivo* is discussed.

Key words: class I histocompatibility antigens/HLA-A2/mutant/RNA splicing/secretion

Introduction

The human class I major histocompatibility antigens (HLA-A, -B and -C) are polymorphic cell surface proteins which mediate interactions between cytotoxic T lymphocytes and virally infected target cells (Zinkernagel and Doherty, 1979; Burakoff, 1981). They are composed of a polymorphic integral membrane glycoprotein heavy chain (44 000 daltons) which is non-covalently associated with an invariant, water soluble light chain, β2-microglobulin (12 000 daltons). Class I heavy chains are encoded by a series of genes within the major histocompatibility complex on human chromosome 6, whereas the light chain is encoded by a gene on chromosome 15 (Ploegh *et al.*, 1981; Steinmetz and Hood, 1983).

One approach to the study of the structure and function of these molecules has been mutant analysis. A series of mutants has been obtained from the B lymphoblastoid cell line T5-1 (HLA-A1, -A2, -B8, -B27) by mutagenesis followed by immunoselection with an HLA-A2 specific monoclonal antibody plus complement (Pious *et al.*, 1982). These mutants all exhibit diminished cell surface expression of the particular HLA-A2 antigenic determinant selected against. In some cases this results from a perturbation of the antigenic structure of the HLA-A2 molecule, while in other cases it results from a reduction in the number of HLA-A2 molecules expressed on the cell surface.

Among the mutants in the latter category, one, clone 8.14.1, displays a particularly novel phenotype (Krangel *et al.*, 1984). This clone synthesizes two distinct forms of the HLA-A2 molecule, a minor form only found associated with the cells, and an abundant form, which is secreted. Whereas the HLA-A2 heavy chain of the cell-associated form is of roughly normal mol. wt., the secreted form is ~4000 – 5000 daltons smaller. Pulse-chase experiments and *in vitro* translation analysis suggest that these species are probably encoded by distinct transcripts. A limited structural analysis comparing the disposition of proteolytic cleavage sites indicates that the secreted molecule differs from the wild-type form in a region near, but not at, the carboxyl terminus of the heavy chain, and that the two forms might be related by an internal deletion.

A segment near the carboxyl terminus of the polypeptide includes a stretch of hydrophobic amino acids thought to interact with the lipid bilayer (Goldman *et al.*, 1979; Ploegh *et al.*, 1981), and is encoded in a distinct, small exon in HLA heavy chain genes (Biro *et al.*, 1982; Malissen *et al.*, 1982; Sodoyer *et al.*, 1984; Strachan *et al.*, 1984; Koller and Orr, 1985). Thus it was proposed that the secreted molecule derived from transcripts spliced so as to delete this exon (Krangel *et al.*, 1984). However, Northern blot analysis failed to reveal a difference between the parent and mutant cell line class I molecule transcripts, and formal proof for this model was lacking. This question is addressed in the present report through the analysis of HLA-A2 encoding cDNA clones obtained from both the parent and mutant cell lines.

Results

cDNA libraries were constructed from poly(A)$^+$ RNA isolated from T5-1 and immunoselected mutant 8.14.1. Approximately 20 000 and 30 000 recombinants, respectively, were screened with an HLA-B7 cDNA probe, and hybridizing clones were detected at a frequency of ~0.7%. Subsets of these clones were analyzed for insert size, and those with inserts of 600 bp or greater were screened to discriminate those which might encode

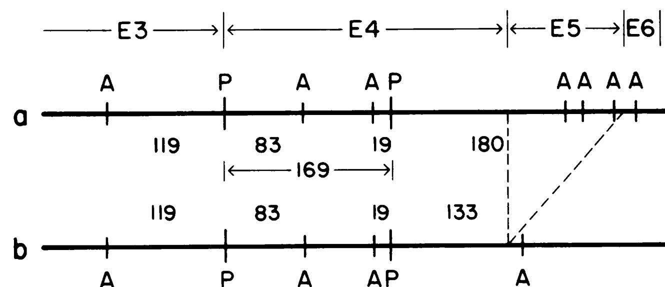

Fig. 1. Predicted partial restriction maps for T5-1 and 8.14.1 HLA-A2 encoding cDNA clones. Map (a) was constructed directly from the sequence of clone JY103 reported by Arnot *et al.* (1984). Map (b) was constructed assuming the precise deletion of exon five. *Pst*I and *Alu*I sites are denoted by (P) and (A), respectively. The sizes of the relevant *Pst*I and *Pst*I-*Alu*I fragments are noted. The boundaries of exons 3 – 6 within this region are denoted by E3, E4, E5 and E6, and the predicted deletion is represented by the dashed line.

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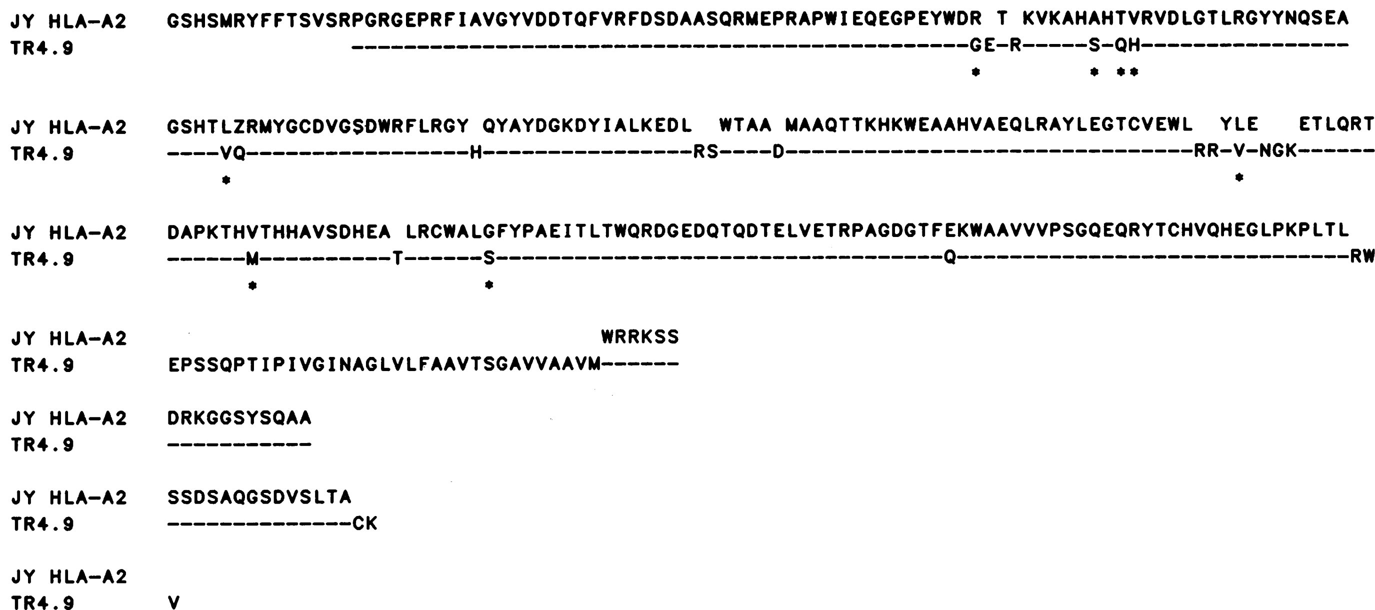

Fig. 2. Comparison between the published protein sequence of HLA-A2 isolated from the JY cell line and the sequence predicted from T5-1 clone TR4.9. Sequences are presented in blocks representing exons two through eight of the 721 HLA-A2 gene (Koller and Orr, 1985). The JY HLA-A2 sequence is derived from Robb *et al.* (1978) and Lopez de Castro *et al.* (1982). Blanks represent unassigned residues. Identity with the protein sequence is denoted by (−). Amino acids are noted which either fill unassigned positions in the protein sequence, or diverge from previous assignments. Significant discrepancies are marked (*).

HLA-A2 molecules. An HLA-A2 related cDNA clone previously obtained from the JY cell line was known to be distinguishable from a number of HLA-B encoding cDNAs by the presence of two *Pst*I sites separated by 169 bp (Arnot *et al.*, 1984; see Figure 1). Eight clones from the T5-1 library and three clones from the 8.14.1 library were found to carry an internal *Pst*I fragment of the appropriate size. Seven of these clones were analyzed further, for the presence of the *Pst*I-*Alu*I fragments predicted from the JY HLA-A2 cDNA sequence (Figure 1). One T5-1 clone, TR4.9, contained all the predicted fragments. One 8.14.1 clone, 14R11.20, was similar except that it lacked the 180-bp fragment predicted to extend 3′ from the second *Pst*I site, and instead contained a 133-bp fragment. However, the 180-bp fragment results from an *Alu*I site situated within exon 5, which was potentially absent from a *bona fide* 8.14.1 HLA-A2 clone. Complete nucleotide sequences of both clones were determined.

Clones TR4.9 and 14R11.20 were 1423 and 629 bp in length, respectively. The translated amino acid sequence of clone TR4.9, from amino acid 15 to the end of the coding region, is compared with the protein sequence determined for JY HLA-A2 (Robb *et al.*, 1978; Lopez de Castro *et al.*, 1982) in Figure 2. There are eight positions at which the cDNA derived sequence diverges from assigned residues in the protein sequence (denoted by asterisks). Further, there are five positions at which the TR4.9 derived amino acid sequence diverges from that predicted from the JY HLA-A2 cDNA sequence (Arnot *et al.*, 1984; not shown), and hence all three sequences are slightly different. The nucleotide sequence of clone TR4.9 is presented in Figure 3. This sequence is identical to the exon sequences of an HLA-A2 genomic clone derived from the cell line 721 (Koller and Orr, 1985). Thus, clone TR4.9 does indeed represent a *bona fide* HLA-A2 encoding cDNA. Although the HLA-A2 molecules derived from two different cell lines, T5-1 and 721, appear to be identical, their precise relationship with the JY HLA-A2 molecule remains an open question.

Figure 3 also contains the nucleotide sequence of clone 14R11.20. This sequence matches exactly the sequence of clone TR4.9, except that a stretch of 117 nucleotides, corresponding precisely to those residues known to derive from exon five (Koller and Orr, 1985), are missing. Thus, the last nucleotide in exon four is contiguous with the first nucleotide in exon six. Since the splice junctions at both ends of exon five occur between the first and second nucleotides of a codon, the reading frame is unaltered. Further, since the last nucleotides of exons four and five are identical, a new codon is not generated at the junction. Hence, based upon the nucleotide sequence of clone 14R11.20, the secreted HLA-A2 molecule synthesized by T5-1 mutant 8.14.1 is missing 39 amino acids, including a short hydrophilic stretch, the transmembrane hydrophobic region, and the positively charged residues immediately carboxy terminal to the hydrophobic region. The remainder of the carboxy terminus, however, is unaltered. This is precisely the structure proposed previously base upon biosynthetic experiments and limited structural data.

Since only a single clone from each library was analyzed in detail, it could not be formally concluded that the difference detected between the clones was actually representative of a significant difference between the wild-type and mutant cell lines, as opposed to a sampling artifact. To address this question, an oligodeoxyribonucleotide (A4/6) was synthesized which was complementary to the coding strand and was comprised of the last 10 nucleotides of exon four juxtaposed with the first 10 nucleotides of exon six. The labeled oligonucleotide was used to probe filters carrying >100 clones from each library which had been selected due to hybridization with the HLA-B7 cDNA probe. Although 11% of the 8.14.1 derived clones stably hybridized with the oligonucleotide, none of the T5-1 derived clones were found to do so. Hence the structures of the cDNA clones selected for study were indeed representative of a significant difference between the two cell lines. Since T5-1 synthesizes four HLA-A and -B locus products in roughly equivalent amounts, and since the majority of the HLA-A2 synthesized by

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                             20                                      30
TR4.9      CCC GGC CGC GGG GAG CCC CGC TTC ATC GCA GTG GGC TAC GTG GAC GAC ACG CAG TTC GTG CGG TTC GAC AGC GAC

           40                                      50                                      60
TR4.9      GCC GCG AGC CAG AGG ATG GAG CCG CGG GCG CCG TGG ATA GAG CAG GAG GGT CCG GAG TAT TGG GAC GGG GAG ACA

                             70                                      80
TR4.9      CGG AAA GTG AAG GCC CAC TCA CAG ACT CAC CGA GTG GAC CTG GGG ACC CTG CGC GGC TAC TAC AAC CAG AGC GAG

           90E2E3                                  100                                     110
TR4.9      GCC GGT TCT CAC ACC GTC CAG AGG ATG TAT GGC TGC GAC GTG GGG TCG GAC TGG CGC TTC CTC CGC GGG TAC CAC

                             120                                     130
TR4.9      CAG TAC GCC TAC GAC GGC AAG GAT TAC ATC GCC CTG AAA GAG GAC CTG CGC TCT TGG ACC GCG GCG GAC ATG GCA
14R11.20   CAG TAC GCC TAC GAC GGC AAG GAT TAC ATC GCC CTG AAA GAG GAC CTG CGC TCT TGG ACC GCG GCG GAC ATG GCA

           140                                     150                                     160
TR4.9      GCT CAG ACC ACC AAG CAC AAG TGG GAG GCG GCC CAT GTG GCG GAG CAG TTG AGA GCC TAC CTG GAG GGC ACG TGC
14R11.20   GCT CAG ACC ACC AAG CAC AAG TGG GAG GCG GCC CAT GTG GCG GAG CAG TTG AGA GCC TAC CTG GAG GGC ACG TGC

                             170                                     180     E3E4
TR4.9      GTG GAG TGG CTC CGC AGA TAC CTG GAG AAC GGG AAG GAG ACG CTG CAG CGC ACG GAC GCC CCC AAA ACG CAT ATG
14R11.20   GTG GAG TGG CTC CGC AGA TAC CTG GAG AAC GGG AAG GAG ACG CTG CAG CGC ACG GAC GCC CCC AAA ACG CAT ATG

           190                                     200                                     210
TR4.9      ACT CAC CAC GCT GTC TCT GAC CAT GAA GCC ACC CTG AGG TGC TGG GCC CTG AGC TTC TAC CCT GCG GAG ATC ACA
14R11.20   ACT CAC CAC GCT GTC TCT GAC CAT GAA GCC ACC CTG AGG TGC TGG GCC CTG AGC TTC TAC CCT GCG GAG ATC ACA

                             220                                     230
TR4.9      CTG ACC TGG CAG CGG GAT GGG GAG GAC CAG ACC CAG GAC ACG GAG CTC GTG GAG ACC AGG CCT GCA GGG GAT GGA
14R11.20   CTG ACC TGG CAG CGG GAT GGG GAG GAC CAG ACC CAG GAC ACG GAG CTC GTG GAG ACC AGG CCT GCA GGG GAT GGA

           240                                     250                                     260
TR4.9      ACC TTC CAG AAG TGG GCG GCT GTG GTG GTG CCT TCT GGA CAG GAG CAG AGA TAC ACC TGC CAT GTG CAG CAT GAG
14R11.20   ACC TTC CAG AAG TGG GCG GCT GTG GTG GTG CCT TCT GGA CAG GAG CAG AGA TAC ACC TGC CAT GTG CAG CAT GAG

                             270                 E4E5                280
TR4.9      GGT TTG CCC AAG CCC CTC ACC CTG AGA TGG GAG CCG TCT TCC CAG CCC ACC ATC CCC ATC GTG GGC ATC ATT GCT
14R11.20   GGT TGG CCC AAG CCC CTC ACC CTG AGA TGG G-- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

           290                                     300                                     310         E5E6
TR4.9      GGC CTG GTT CTC TTT GGA GCT GTG ATC ACT GGA GCT GTG GTC GCT GCT GTG ATG TGG AGG AGG AAG AGC TCA GAT
14R11.20   --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- -AT

                             320                 E6E7                330
TR4.9      AGA AAA GGA GGG AGC TAC TCT CAG GCT GCA AGC AGT GAC AGT GCC CAG GGC TCT GAT GTG TCT CTC ACA GCT TGT
14R11.20   AGA AAA GGA GGG AGC TAC TCT CAG GCT GCA AGC AGT GAC AGT GCC CAG GGC TCT GAT GTG TCT CTC ACA GCT TGT

           340E7E8
TR4.9      AAA GTG TGA GACAGCTGCCTTGTGTGGGACTGAGAGGCAAGAGTTGTTCCTGCCCTTCCCTTTGTGACTTGAAGAACCCTGACTTTGTTTCTGCAA
14R11.20   AAA GTG TGA GACAGCTGCCTTGTGTGGGACTGAGAGGCAAGAGTTGTTCCTGCCCTTCCCTTTGTGACT

TR4.9      AGGCACCTGCATGTGTCTGTGTTCGTGTAGGCATAATGTGAGGAGGTGGGGAGACCACCCCACCCCCATGTCCACCATGACCCTCTTCCCACGCTGACC

TR4.9      TGTGCTCCCTCCCCAATCATCTTTCCTGTTCCAGAGAGGTGGGGCTGAGGTGTCTCCATCTCTGTCTCAACTTCATGGTGCACTGAGCTGTAACTTCTT

TR4.9      CCTTCCCTATTAAAATTAGAACCTGAGTATAAATTTACTTTCTCAAATTCTTGCCATGAGAGGTTGATGAGTTAATTAAAGGAGAAGATTCCTAAAATT

TR4.9      TGAGAGACAAAATAAATGGAACACATGAGAAAAAAAAAAAAAAAA
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Fig. 3. Comparison between the nucleotide sequences determined for T5-1 clone TR4.9 and 8.14.1 clone 14R11.20. Sequences within the coding regions are divided into codons which are numbered from the amino terminus of the mature wild-type molecule. (−) indicates nucleotides encoded in exon five, which are absent from clone 14R11.20. Exon boundaries are marked. The nucleotides immediately adjacent to the linker at the end of each clone corresponding to the 5′ end of the mRNA are not reported, since artifacts are commonly found in the region of the hairpin loop.

mutant 8.14.1 is of the secreted variety, one might predict that ~20−25% of the class I cDNA clones in the 8.14.1 library should derive from mRNA encoding the secreted molecule. That A4/6 hybridizing clones were detected at half this rate is not surprising, since the average insert size of the library was such that many clones derived from the appropriate mRNA species probably lacked the sequence being probed.

As noted previously, it has not been possible to distinguish T5-1 and 8.14.1 class I antigen mRNA by agarose gel electrophoresis followed by Northern blot analysis. This is demonstrated in Figure 4, panel A, in which the HLA-B7 cDNA has been used as a probe. However, when the same filter is probed with A4/6, a strikingly different result is obtained (Figure 4, panel B). In this case hybridization is detected to transcripts

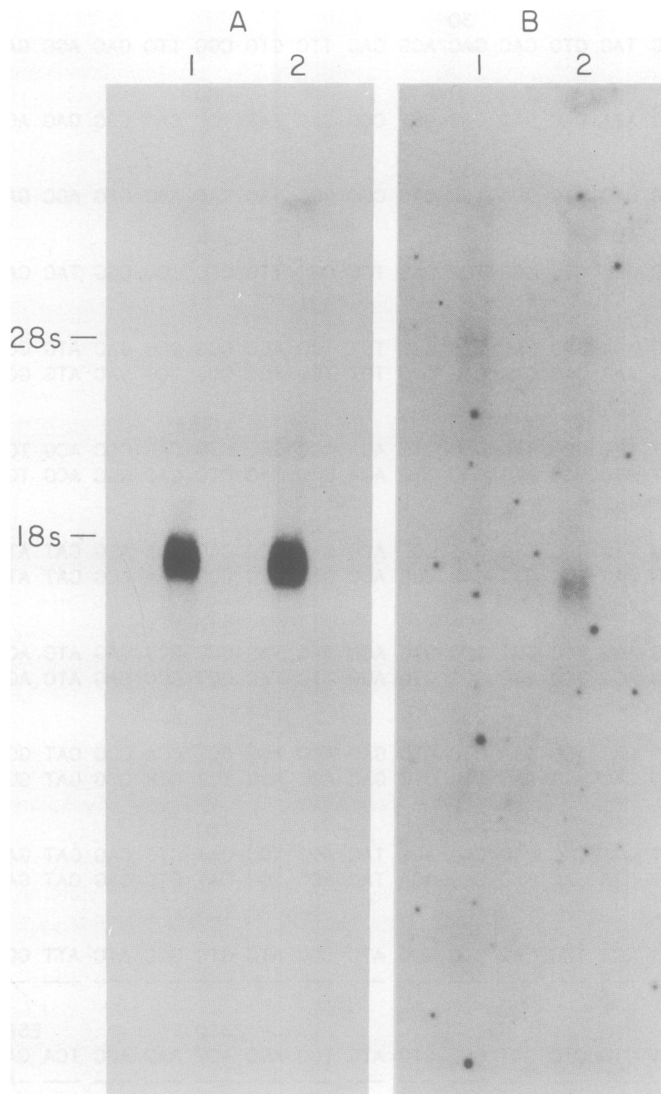

Fig. 4. Northern blot analysis of T5-1 and 8.14.1 RNA. Poly(A)$^+$ RNA isolated from T5-1 (**lane 1**) and 8.14.1 (**lane 2**) was fractionated on 1.5% agarose gels, transferred to nitrocellulose, and hybridized with either the HLA-B7 cDNA probe (**panel A**) or oligonucleotide probe A4/6 (**panel B**), as described in Materials and methods. The same nitrocellulose filter was used with both probes.

in 8.14.1 RNA, but not T5-1 RNA, which are of slightly greater average mobility than the bulk of the class I antigen transcripts. That 8.14.1-specific, A4/6 hybridizing transcripts are only detected at this position implies that the failure to include exon five is not associated with any further derangement of transcript processing. Some hybridization is observed to higher mol. wt. species present in both cell lines. This probably represents residual non-specific hybridization to both 28S and 18S rRNA, which contaminated the oligo(dT)-selected RNA used in this experiment.

To determine whether the loss of exon five from 8.14.1 HLA-A2 transcripts was due to a deletion of this sequence from the HLA-A2 gene, *Hind*III-digested genomic DNAs from the parent and mutant cell lines were electrophoresed on agarose gels and compared by Southern hybridization analysis. When using the HLA-B7 cDNA as a probe, 12 bands are resolved and visualized; however, no difference between the two cell lines can be

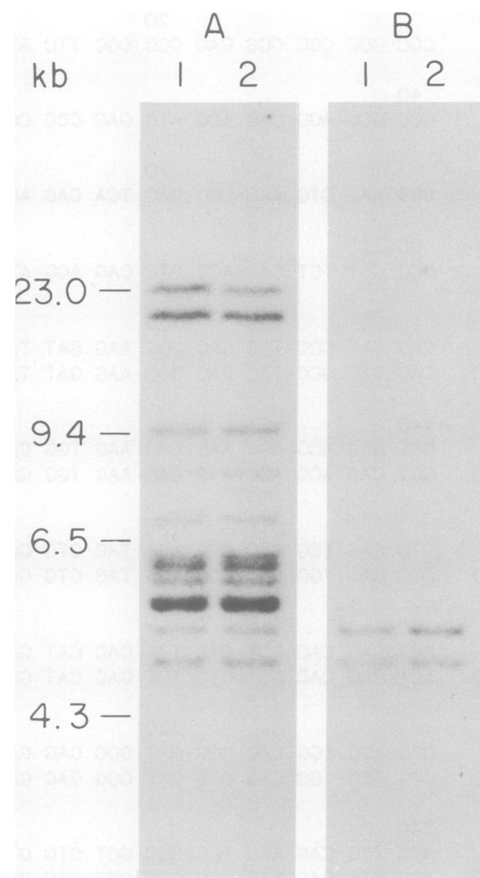

Fig. 5. Southern blot analysis of T5-1 and 8.14.1 genomic DNA. High mol. wt. DNA isolated from T5-1 and 8.14.1 was digested with *Hind*III, electrophoresed on 0.7% agarose gels and transferred to nitrocellulose as described in Materials and methods. Nitrocellulose filters were probed with either the HLA-B7 cDNA (**panel A**) or with clone pHLA-2a.1 (**panel B**). **Lane 1**: T5-1 digest; **lane 2**: 8.14.1 digest.

discerned (Figure 5, panel A). A clone described by Koller *et al.* (1984) (pHLA-2a.1), derived primarily from the 3'-untranslated region of an HLA-A2 genomic clone, will preferentially hybridize to HLA-A locus derived restriction fragments at the appropriate stringency of washing. When using pHLA-2a.1 as a probe and washing at high stringency, two fragments of 4.6 and 5.0 kb were preferentially visualized (Figure 5, panel B). Similarly sized fragments have been shown to carry the HLA-A1 and HLA-A2 genes, respectively, in the genomic DNA of the 721 cell line (Koller *et al.*, 1984). Furthermore, the smaller fragment has been shown to correlate precisely with HLA-A1 in population studies (Orr and DeMars, 1983). Since T5-1, like 721, expresses both HLA-A1 and HLA-A2, it can be concluded that the T5-1 HLA-A2 gene resides on the 5.0-kb *Hind*III fragment. A deletion in this fragment of 117 bp or greater could have been detected. Further, there is no evidence for a second HLA-A2 gene in 8.14.1. Hence, at this level of resolution, the HLA-A2 genes in the two cell lines are indistinguishable. Thus the sequences absent in transcripts encoding the secreted HLA-A2 molecule are not simply deleted from the 8.14.1 HLA-A2 gene, and the mutation in 8.14.1 is probably a limited one which affects the relative use of exon five and exon six splice sites. This is consistent with the observation that at low levels 8.14.1 synthesizes an HLA-A2 species of roughly normal mol. wt., which remains cell associated at all times.

Discussion

Previous work has identified a T5-1 mutant, 8.14.1, which, unlike the parent cell line, synthesizes both a cell-associated and a secreted form of HLA-A2 (Krangel *et al.*, 1984). Structural studies demonstrated that the secreted form is distinguishable from wild-type by the apparent loss of a 4000 – 5000 dalton segment of the polypeptide situated near, but not at, the carboxy terminus of the molecule. Further, both forms of HLA-A2 appear to be primary translation products. Based upon these observations as well as the known intron/exon structure of class I antigen genes, it was proposed that the secreted molecule derives from unusually spliced HLA-A2 transcripts which are missing exon five, and hence lack the hydrophobic segment which would normally anchor the polypeptide in the plasma membrane. The present study reports the isolation of cDNA clones for both the wild-type HLA-A2 molecule synthesized by T5-1 cells and the secreted HLA-A2 molecule synthesized by mutant 8.14.1. A comparison of their structures indicates that the latter is identical to the former save for the precise deletion of the 117-bp region encoded by exon five. Analysis of *Hind*III-digested genomic DNA from T5-1 and mutant 8.14.1 on Southern blots indicates the presence of only a single 8.14.1 HLA-A2 gene, and suggests that a deletion the size of exon five has not occurred. These results offer strong support to the alternative splicing model invoked to explain the unusual phenotype of 8.14.1.

The exact nature of the mutation in 8.14.1 cells has not been defined. HLA-A2 is the only allele affected (Krangel *et al.*, 1984) and hence the mutation presumably maps to the HLA-A2 gene itself. However, except for the 117-bp deletion, no other differences distinguish the two clones examined. Since a cDNA clone encoding the lower abundance, membrane form of HLA-A2 synthesized by 8.14.1 cells was not identified, its precise structure is not formally known. However, since this polypeptide is of roughly normal mol. wt., and remains associated with 8.14.1 cells at all times, it is presumably encoded by transcripts which retain exon five. This would imply that exon five has not been deleted from the 8.14.1 HLA-A2 gene, a conclusion supported by Southern blot analysis. Although ethylmethane sulfonate, which was used to mutagenize T5-1 cells, can induce deletions (D.Pious, personal communication), it primarily induces point mutations. Thus it seems likely that the primary lesion in 8.14.1 is a limited mutation which affects the relative use of the exon five and exon six splice acceptor sites. Such a mutation might be localized within the exon five splice acceptor or donor consensus sequences, or possibly at some other sequence required for efficient splicing at either of these sites. Hence this mutation may be in some ways analogous to those which affect globin pre-mRNA splicing in a number of thalassaemias (Treisman *et al.*, 1983). Of course, the precise definition of the mutation in 8.14.1 requires the analysis of HLA-A2 genomic clones from both parent and mutant cell lines.

It appears to be a general finding, but not a universal one, that an integral membrane protein can be converted into a stable, secreted form by removal of the membrane anchoring portion of the polypeptide. For example, secreted forms of influenza hemagglutinin (Gething and Sambrook, 1982), VSV G protein (Rose and Bergmann, 1982), HSV gC protein (Holland *et al.*, 1984), the human EGF receptor (Mayes and Waterfield, 1984; Ullrich *et al.*, 1984), and the human LDL receptor (Lehrman *et al.*, 1985) can be generated by mutations which result in the synthesis of a truncated polypeptide. However, a truncated form of RSV gp37 fails to be secreted (Willis *et al.*, 1984). The factors involved in determining the fate of such proteins are not understood. However it is reasonable to assume that secretion requires that the extracellular portion of the polypeptide constitute an independent structural domain. This is indeed the case for class I histocompatibility antigens, since proteases can be used to cleave the extracellular portion off the membrane in a stable, antigenically active form.

There exist numerous reports in the literature which describe soluble and/or serum forms of class I molecules (Miyakawa *et al.*, 1973; Natori *et al.*, 1976; Billing *et al.*, 1977; Kvist and Peterson, 1978; Emerson and Cone, 1981; Ramanathan *et al.*, 1982; Kress *et al.*, 1983a; Maloy *et al.*, 1984; Pellegrino *et al.*, 1984). The example which has been best characterized as to structure and origin is that of a murine Qa region gene product which is synthesized only in the liver, and can be detected in the serum (Cosman *et al.*, 1982; Kress *et al.*, 1983a; Maloy *et al.*, 1984; Mellor *et al.*, 1984). The gene encoding this molecule is distinct from other class I genes studied so far in that exon five contains numerous substitutions, as well as a deletion which results in a frame shift and a termination codon. Although cDNA clones encoding this molecule appear to lack exon seven, the secretion of this molecule is undoubtedly the result of the various changes in exon five, which is included in the mature transcript. Hence this mechanism is clearly distinct from that reported here for the HLA-A2 molecule secreted by 8.14.1 cells.

Although serum forms of class I molecules antigenically related to HLA-A and -B locus products have been detected, their precise structure and orgin have not been characterized (Miyakawa *et al.*, 1973; Billing *et al.*, 1977; Pellegrino *et al.*, 1984). Thus, it is unclear by what mechanism they arise. The analysis of class I antigen cDNA clones has demonstrated alternative splicing to be operative in the choice of splice donor and acceptor sites at the 5′ end of exon two (Transky *et al.*, 1984), in the removal of exon seven (Brickell *et al.*, 1983), and in the choice of splice acceptor sites within exon eight (Kress *et al.*, 1983b). Furthermore, transcripts of different class I genes may be preferentially spliced in one fashion or another (discussed in Hood *et al.*, 1983; Kress *et al.*, 1983b). However, a natural example of a class I antigen encoding cDNA clone which lacks exon five has not been described. The results presented in this and a preceding paper (Krangel *et al.*, 1984) indicate that information encoded within exon five is essential for anchoring class I molecules in the plasma membrane, and that when this exon is absent from class I antigen transcripts, a stable, secreted molecule is synthesized which is antigenically related to the parent membrane molecule. Whether this process actually occurs *in vivo*, and what its physiological significance would be, remain open questions.

Materials and methods

cDNA library construction and screening

One liter cultures of T5-1 and 8.14.1 cells were used to prepare total RNA by the method of Auffray and Rougeon (1980). One round of chromatography on oligo(dT)-cellulose was used to enrich for poly(A)-containing RNA (Aviv and Leder, 1972). Double-stranded cDNA was synthesized as described (Helfman *et al.*, 1983), with the sequential addition of *Eco*RI and *Sal*I linkers, and was size fractionated by chromatography on Sepharose 4B. Molecules 400 bp or longer were ligated into *Eco*RI and *Sal*I cut pBR322, which was used to transform *Escherichia coli* strain DH1 as described (Hanahan, 1983). Ampicillin-resistant colonies were selected, replicated, amplified and screened on nitrocellulose filters as described (Hanahan and Meselson, 1980). An HLA-B7 cDNA clone insert (Sood *et al.*, 1981) was nick-translated (Maniatis *et al.*, 1975) and used to probe the filters at 42°C in 50% formamide, 1 x Denhardt's solution, 3 x SSC, 160 μg/ml salmon sperm DNA, 50 mM Hepes, pH 7.0. Filters were washed with four changes of 0.1 x SSC, 0.1% SDS over the course of 2 h at 50°C, and were exposed to X-ray film.

Identification of HLA-A2 encoding cDNA clones

Plasmid minipreps were prepared from positive colonies by a boiling procedure (Holmes and Quigley, 1981). Plasmid DNA was digested with either *Eco*RI and *Sal*I to check insert size, or with *Pst*I to assess the presence of an internal *Pst*I fragment of the appropriate size. Digested DNA was resolved in 1.5% agarose gels, using ϕX *Hae*III fragments as markers. Plasmid preps (Maniatis *et al.*, 1982) prepared from selected colonies were used to analyze *Pst*I-*Alu*I fragments. Plasmid DNA was digested with *Pst*I, end-labeled using T4 DNA polymerase (Maniatis *et al.*, 1982) in the presence of 0.4 mM dATP and dTTP, and 200 μCi/ml [^{32}P]-dGTP and dCTP, and was then redigested with *Alu*I. Radiolabeled fragments were resolved on 25 cm long 8% polyacrylamide gels.

DNA sequence analysis

14R11.20 plasmid DNA was digested with *Eco*RI and *Sal*I and the Klenow fragment of *E. coli* DNA polymerase was utilized to produce blunt ended molecules. These were either cloned directly into the *Sma*I site of M13mp8 or were further digested with *Pst*I and then incubated with T4 DNA polymerase to produce blunt ended molecules, prior to cloning. TR4.9 insert DNA was digested with *Pst*I and *Pvu*II and the resulting fragments were incubated with T4 DNA polymerase to produce blunt ended fragments which were then cloned into the *Sma*I site of M13mp8. Alternatively TR4.9 plasmid DNA was linearized utilizing *Kpn*I and cloned into the *Kpn*I site of M13mp18. Fragments were sequenced by the dideoxy-chain termination method (Sanger *et al.*, 1977), utilizing 6% polyacrylamide gels with and without buffer gradients (Biggin *et al.*, 1983).

Blot hybridization analysis

The isolation of high mol. wt. DNA from T5-1 and 8.14.1 cells was essentially as described (Maniatis *et al.*, 1982). 50 μg DNA was digested by three additions of 50 U *Hin*dIII spaced over a 24 h incubation at 37°C. DNA (10 μg/lane) was electrophoresed at 50 V through 23 cm long 0.7% agarose gels in the presence of 45 mM Tris-borate, 1 mM EDTA, pH 8.2. *Hin*dIII-digested lambda DNA size markers were visualized by ethidium bromide staining. Transfer to nitrocellulose was by the method of Southern (1975). Inserts isolated from the HLA-B7 cDNA clone and clone pHLA-2a.1 (Koller *et al.*, 1984) were labeled by nick-translation and used as hybridization probes at 3 x 10^6 c.p.m./ml under conditions described above. Filters were washed initially with 1 x SSC, 0.5% SDS at 20°C for 30 min, and then with 0.1 x SSC at either 50°C (HLA-B7) or 68°C (pHLA-2a.1) for 1 h, with four changes. Oligo(dT)-selected RNA was resolved by electrophoresis at 60 V through 16 cm long 1.5% agarose gels containing 2.2 M formaldehyde as described (Lehrach *et al.*, 1977), and was transferred to nitrocellulose according to Thomas (1980). When utilizing the HLA-B7 cDNA as a probe, conditions for hybridization and washing were identical to those described for Southern filters except that the washes were performed at 42°C instead of 50°C.

Use of the synthetic oligodeoxyribonucleotide

The A4/6 20-mer 5'-CCTTTTCTATCCCATCTCAG-3', was kindly synthesized by Mark Zoller. It was labeled with [^{32}P]ATP and T4 polynucleotide kinase as described (Maniatis *et al.*, 1982), and the labeled 20-mer was separated from unincorporated ATP by chromatography through Sephadex G-25 in 10 mM NH_4HCO_3. Filters carrying cDNA clones were pre-hybridized in 6 x SSC, 10 x Denhardt's solution and 0.2% SDS for 2 h at 68°C and then hybridized at 1 x 10^6 c.p.m./ml in 6 x SSC and 10 x Denhardt's solution for 12 h at 42°C. Filters were washed in three 15 min changes of 6 x SSC at 23°C, and were then washed for 2 min in 6 x SSC at 48°C and 2 min in 6 x SSC at 65°C. For Northern filters, SDS was not included in the pre-hybridization solution, hybridization was carried out with 2 x 10^6 c.p.m./ml at 23°C, and the 23°C washes were followed by two washes for 2 min each in 6 x SSC at 57°C.

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