The EMBO Journal vol.4 no.11 pp.2849–2854, 1985

Exon shuffling *in vivo* can generate novel HLA class I molecules

Nicholas Holmes and Peter Parham

Department of Cell Biology, Stanford University School of Medicine, Stanford, CA 94305, USA

Communicated by P.N.T.Unwin

The human class I histocompatibility molecule HLA-Aw69 has serological and structural properties which suggested it was a hybrid of the allelic products HLA-A2 and HLA-Aw68. We have now isolated three genes for HLA-Aw69 and one gene for HLA-Aw68. The sequences of exons encoding the entire extracellular portion of the molecule and of intron 2 have been determined. Their comparison with the published sequence of HLA-A2 proves that HLA-Aw69 is a hybrid molecule with complete identity to HLA-Aw68 in the α_1 domain and with HLA-A2 in the α_2 and α_3 domains. This comparison also localised regions involved in the epitopes recognised by monoclonal antibodies. The three HLA-Aw69 genes obtained from unrelated individuals of diverse ethnic backgrounds are identical. All results are consistent with HLA-Aw69 having arisen by a single reciprocal recombination event between the HLA-Aw68 and HLA-A2 genes somewhere in a region of 86 bp about the 3′ donor splice site of exon 2. Estimates of the silent mutation rate in HLA genes suggest this event occurred not more than 330 000 years ago. Intra-allelic reciprocal recombination thus represents a further mechanism in addition to gene conversion for the generation of novel class I histocompatibility alleles.

Key words: class I histocompatibility genes/HLA-Aw69/recombination/evolution/hybrid genes

Introduction

The major histocompatibility (MHC) class I molecules consist of a family of highly polymorphic membrane glycoproteins. They serve as cellular co-recognition signals for the activation of cytotoxic T lymphocytes directed against many target antigens. Their polymorphism is known to play an important role in determining immune responsiveness in mice and has been implicated in human immune responses.

The class I molecules are composed of a polymorphic MHC-encoded heavy chain of 44 000 daltons non-covalently associated with β_2-microglobulin (reviewed by Ploegh *et al.*, 1981). MHC class I heavy chains possess three external protein domains (α_1, α_2, α_3) of ~90 amino acids in length, a transmembrane segment and a small cytoplasmic region. The α_3 domain and β_2 microglobulin share strong homology to immunoglobulin domains and are well conserved within a species (reviewed by Kimball and Coligan, 1983). Almost all the polymorphic variation is found in the α_1 and α_2 domains.

Comparison of the primary structures of class I molecules has been undertaken in order to discern patterns of polymorphic variation and evolutionary relationships. One group of related molecules which has been studied focuses on the HLA-A2 and A28 molecules. Several members of this family are now recognised, some of which are serologically distinguishable (Russo *et al.*, 1983; Albert *et al.*, 1984), and others can only be discriminated by cytotoxic T lymphocytes (Biddison *et al.*, 1982; Van der Poel *et al.*, 1983; Gotch *et al.*, 1985). We have studied the relationship between three alleles belonging to the HLA-A2, A28 family. HLA-A2.1 (Van der Poel *et al.*, 1983) is the predominant HLA-A2 allele present in the Caucasian population hereafter referred to simply as HLA-A2. HLA-Aw68 and HLA-Aw69 are two subtypes of HLA-A28. All three molecules are distinguishable by reactivity with panels of monoclonal antibodies (Parham *et al.*, 1982). Furthermore we have shown by tryptic peptide mapping that the products of these three alleles are structurally distinct (Holmes and Parham, 1984). From the peptide data we formed the hypothesis that the HLA-Aw69 allele had arisen as a result of a genetic exchange event involving HLA-Aw68 and HLA-A2 genes.

To test this hypothesis and to define accurately the relationship between HLA-A2, HLA-Aw68 and HLA-Aw69 we have cloned the genes for HLA-Aw69 and HLA-Aw68 and compared their sequences with HLA-A2 (Koller and Orr, 1985). In addition we have compared the sequences of three HLA-Aw69 genes isolated from unrelated and ethnically diverse individuals.

Results

Identification and expression of the HLA-A locus genes

Partial genomic libraries were made from three cell lines: IDF, BJ and ZM expressing HLA-Aw69 and one cell line, LB, expressing HLA-Aw68. (Hereafter all HLA-A, B, C genes and molecules will be referred to by the number alone, e.g., HLA-Aw68 is Aw68.) Putative HLA-A locus genes were identified by screening $0.5-1 \times 10^6$ recombinant phage with both a B7 cDNA probe that hybridized to all class I genes and an HLA-A locus-specific probe, pHLA-2a.1 (Koller *et al.*, 1984). The number of plaques which hybridized to the pHLA-2a.1 probe was 8–14 per 10^6 phage screened. The B7 cDNA probe gave 2–4 times as many positive reactions as the pHLA-2a.1 probe. For those libraries that were amplified before screening the frequency of positive plaques with both probes was increased by ~10-fold, demonstrating that the phage containing class I inserts were selectively amplified.

Table I. The identification of Aw68 and Aw69 genes by expression in mouse L cells

	Transfected clone	c.p.m.[^{125}I]RAM bound			Gene assignment
		CR11-351	W6/32	MA2.1	
Expt. 1	LBA5	12 262	14 485	1353	Aw68
	Ltk$^-$	1849	1593	1760	
Expt. 2	IDFA1	12 989	12 022	1027	Aw69
	BJA1	9189	10 564	729	Aw69
	ZMA1	11 310	6127	646	Aw69
	Ltk$^-$	442	577	550	

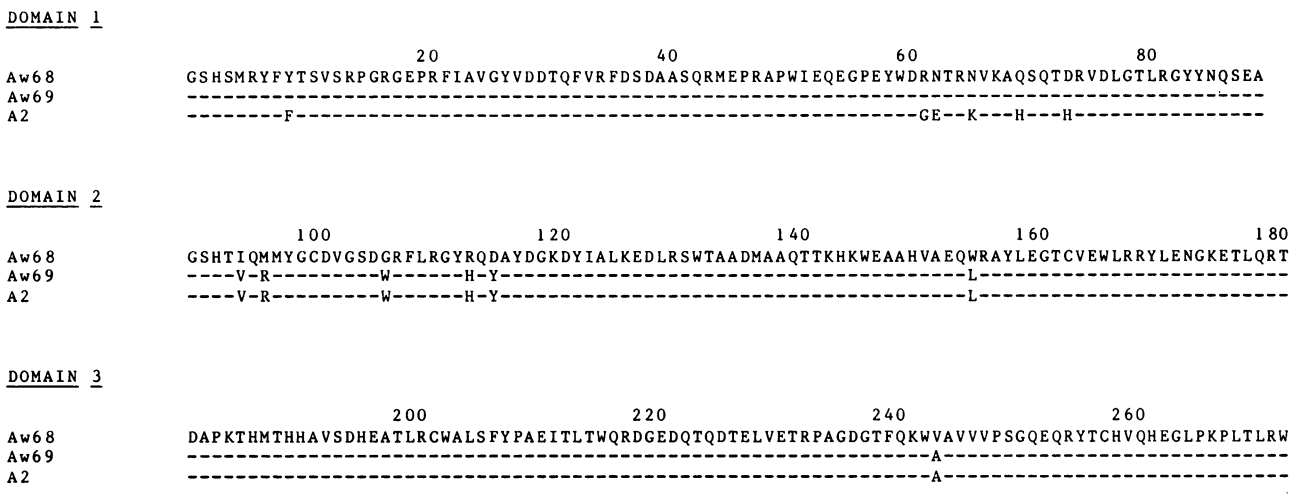

Fig. 1. Comparison of the deduced protein sequences for the first 274 residues of HLA-Aw68 from the genomic clone LBA5 of HLA-Aw69 from the genomic clones IDFA1, BJA1 and ZMA1 and of HLA-A2 from the LCL 721 cell line (Koller and Orr, 1985). Identity of HLA-Aw69 and/or HLA-A2 with HLA-Aw68 is denoted by (-). The standard one-letter amino acid code is used.

Phage plaques hybridizing to both probes were purified to homogeneity. DNA prepared from these clones were analyzed by restriction endonuclease cleavage and by Southern blotting with both hybridization probes. The LB and IDF libraries were the first to be screened and two types of insert were found in the purified clones. All the clones contained an *Eco*RI insert of 6.5 kb but they formed two groups according to the size of their internal *Hind*III fragments which were 5.4 kb and 5.1 kb, respectively. Quantitative DNA hybridization at 68°C, 0.1 × SSC showed that the 5.4-kb *Hind*III inserts were less homologous to the pHLA-2a.1 probe than the 5.1-kb *Hind*III inserts. Since the pHLA-2a.1 probe was derived from the HLA-A2 gene and as the A2 gene is also found as a 5.1-kb *Hind*III fragment (Koller *et al.*, 1984), this suggested that the 5.1-kb fragments contained HLA-A locus genes and that the 5.4-kb fragments may contain another class I gene. This was confirmed by DNA-mediated gene transfer as described below. In subsequent experiments to analyze the BJ and ZM libraries, conditions of higher stringency (68°C, 0.1 × SSC) were used and this permitted the discrimination of the two types of clones during plaque purification. The class I genes found on the 5.4-kb *Hind*III fragments from LB and IDF were not investigated further.

Unambiguous assignment of cloned class I genes can only be made by identifying the products expressed by those genes. Thus the putative HLA-A locus genes obtained from all four libraries were introduced into mouse Ltk$^-$ cells together with the *Tk* gene as a selectable marker. HAT resistant cells were grown as bulk cultures and screened by indirect radioassay with three monoclonal antibodies: CR-11-351 that is specific for A2, Aw68 and Aw69; MA2.1 that binds A2 but not Aw68 and Aw69 and W6/32 that binds all HLA-A, B, C molecules. Table I shows the results of two assays which permitted identification of one HLA-Aw68 gene (clone LBA5) and three HLA-Aw69 genes (clones IDFA1, BJA1, ZMA1, respectively).

Sequence analysis

The 5.1-kb *Hind*III fragments containing HLA-Aw68 and HLA-A69 genes were subcloned into M13mp19 in both orientations. Specific oligonucleotide primers were used to obtain the sequence of exons 2, 3 and 4 and of intron 2. The amino acid sequences deduced from these data are shown in Figure 1 and compared with the published sequence of HLA-A2 (Koller and Orr, 1985). The deduced sequence of Aw68 agrees well with the partial protein sequence of this molecule also obtained from the LB cell line (Lopez de Castro *et al.*, 1982). The single difference is that residue 207 is predicted to be a serine rather than the glycine assigned by the protein sequencing. The nucleotide sequence also identifies 19 previously unassigned residues.

In comparing the sequences of Aw68 with A2 we found 22 nucleotide changes; 11 in exon 2, three in intron 2, seven in exon 3 and one in exon 4. When calculated by the method of Perler *et al.* (1980) these changes consist of five silent, 14 replacement and three non-coding substitutions. This leads to estimates for the amount of silent substitution of 0.455% and replacement substitution of 0.469% between the Aw68 and A2 exons compared. These nucleotide substitutions result in 13 amino acid substitutions between A2 and Aw68 out of 274 residues in the three extracellular domains. It is striking that most of these differences are clustered into two regions previously recognised as especially polymorphic (Lopez de Castro *et al.*, 1982; Ways *et al.*, 1985). Five amino acid differences are found between residues 62 and 74 and five between 95 and 116.

Inspection of the sequences in Figure 1 clearly shows that Aw69 is identical to Aw68 in the first extracellular domain (α_1) and to A2 in the second (α_2) and third (α_3) extracellular domains. The identity exists at both the amino acid (Figure 1) and nucleotide levels (Figure 2) and strongly suggests that the Aw69 gene was formed through a genetic exchange event involving allelic A2 and Aw68 genes. The simplest event that could produce the observed result is a single recombination in the region between exons 2 and 3. These results are entirely consistent with the results and interpretations of our previous tryptic peptide map analysis (Holmes and Parham, 1984).

To determine more precisely the point of possible recombination we sequenced intron 2 that separates exons 2 and 3. The nucleotide sequences of A2, Aw68 and Aw69 are shown in Figure 2 and were analyzed to find at which point the Aw69 gene sequence changes from being identical to Aw68 to being identical to A2. Position 219 in exon 2 is the most 3′ position at which Aw69 is identical to Aw68 and different from A2. Conversely, position 306 in intron 2 is the most 5′ position at which Aw69 is identical to A2 and different from Aw68. We can therefore localise the cross-over point to within a region of 86 bp that encompasses 51 bp 5′ and 35 bp 3′ of the exon 2 donor splice junc-

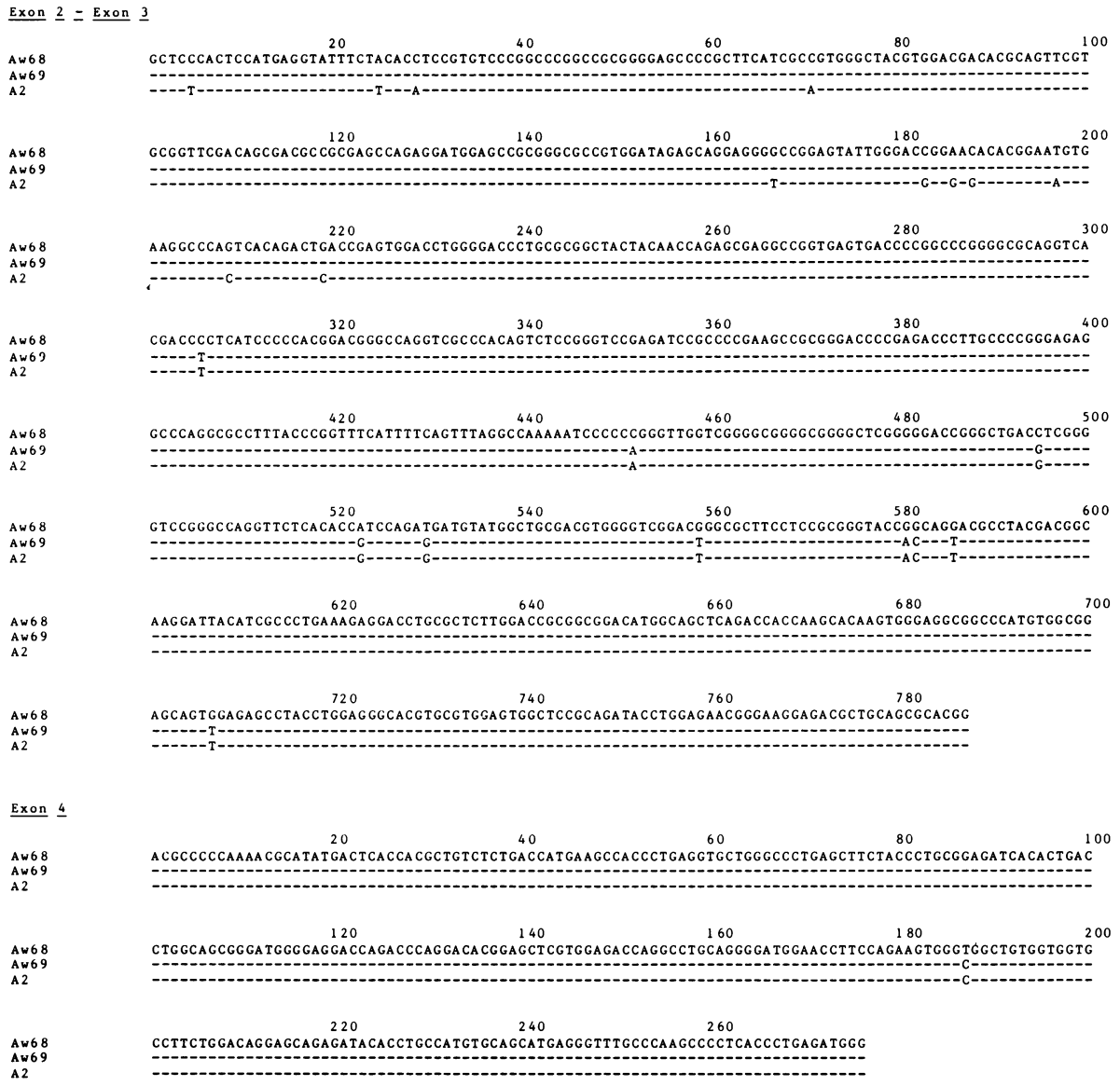

Fig. 2. Comparison of the partial nucleotide sequences of HLA-Aw68 (LB cell line) and HLA-Aw69 (IDF, BJ and ZM cell lines) with the published HLA-A2 sequence (LCL 721 cell line; Koller and Orr, 1985). The sequences of exon 2-intron 2-exon 3 and of exon 4 are shown. Identity with HLA-Aw68 is denoted by (-).

tion. These data are summarized schematically in Figure 3. Aw69 is shown as identical to Aw68 at its 5′ end (stippled region) and identical to A2 at its 3′ end (open region with bars indicating nucleotide differences). The apparent recombination point is also shown in Figure 3.

To define the extent of the recombination more clearly we sequenced the Aw68 and Aw69 genes on a single DNA strand extending 5′→3′ from the 5′ *Hind*III site. The results show that Aw68 and Aw69 are identical for at least 272 nucleotides 3′ of the *Hind*III site and that both genes differ from A2 at eight positions within this region (data not shown).

The analysis described above was carried out on three Aw69 genes, isolated from different cell lines that were chosen for their differences in ethnic origin. The donors of blood used to make the IDF, BJ and ZM cell lines were respectively European Jewish, Black American and Mexican American. In those regions of the genes that were analyzed total identity can be observed and for this reason single Aw69 amino acid and nucleotide sequences are shown in Figures 1 and 2. These results support the interpretation that fixation after a single recombination event was responsible for the origin of all three genes and that no subsequent mutations have occurred, either in the donor genes, A2 and Aw68 or the three recombinant Aw69 genes we analyzed.

To provide an estimate of the time which has elapsed since the proposed recombination, we calculated the maximum percentage silent divergence within silent and non-coding sequences allowed by our observations that no substitutions were observed in 240 non-coding and 299 silent sites. Using the adjusted figures for the rates of divergence in MHC genes of Hayashida and Miyata (1983) of 3.1×10^{-9} and 2.5×10^{-9} replacements per site per year for silent sites and short introns, respectively, it can be calculated that the Aw69 genes are less than ~670 000 years apart. If, however, we include the data from the 5′-flanking sequence, the maximum is ~330 000 years.

Localization of antigenic determinants

In addition to information on their evolution, comparison of the sequences in Figure 2 provides further understanding of the

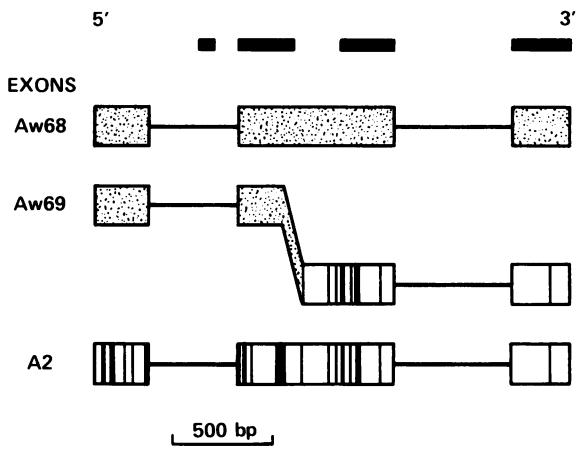

Fig. 3. The recombinant phenotype of HLA-Aw69 is indicated diagrammatically. Regions of the gene which were sequenced are depicted by boxes. HLA-Aw68 sequences are stippled. HLA-A2 (from Koller and Orr, 1985) is shown by open boxes in which solid lines indicate the positions of nucleotide differences with HLA-Aw68. The positions of exons 1, 2, 3 and 4 are indicated above by solid bars. HLA-Aw69 is identical to HLA-Aw68 at its 5' end, as indicated by the stippled region, and to HLA-A2 at its 3' (denoted by open boxes with bars). The region of cross-over is shown schematically.

chemical basis for two polymorphic antigenic determinants of the HLA-A2 family of molecules.

As PA2.1 and similar monoclonal antibodies bind A2 and Aw69 but not Aw68 we can conclude that polymorphic residues in the α_2 domain, which is shared by A2 and Aw69 but not Aw68, are critical for the formation of this epitope. In the α_2 domain A2 and Aw69 differ from Aw68 in six positions, five of which are clustered in the region from 95 to 116. At two positions, valine 95 and tryptophan 107, A2 and Aw69 share residues that are not found in other HLA-A, B, C molecules sequenced making them the best candidates for formation of the A2, Aw69-specific epitope.

MA2.1 and similar monoclonal antibodies are specific for A2 and B17 and do not recognise Aw68 and Aw69. As A2 shares the α_2 domain with Aw69 we can conclude that it is polymorphic residues in the α_1 domain that are critical for formation of MA2.1 epitope. In the α_1 domain A2 differs from Aw68 and Aw69 at six residues, five of which are clustered between positions 62 and 74 and these provide candidates for involvement in the MA2.1 epitope. Again these residues lie in a region of α_1 that has been recognized as being particularly polymorphic. This interpretation is consistent with the results of a comparison of the sequence of A2 with one of the subtypes of B17 (Bw58) which implicated residues 62 – 65 in the formation of the MA2.1 epitope (Ways *et al.*, 1985).

Discussion

The HLA-Aw69 molecule was originally detected as a variant, called A28* or A2/A28, of HLA-A28 which bound monoclonal antibodies that were otherwise specific for HLA-A2 (Brodsky *et al.*, 1979; Parham and Brodsky, 1981). Further serological analysis showed that Aw69 is widespread in human populations and it was given an official HLA-A designation at the Ninth International Histocompatibility Workshop (Albert *et al.*, 1984). Previous comparison of A2, Aw68 and Aw69 molecules by tryptic peptide mapping (Holmes and Parham, 1984) and isoelectric focusing (Antonelli *et al.*, 1985) showed that Aw69 is a chemically distinct molecule. Furthermore, the peptide maps suggested that Aw69 was a hybrid of A2 and Aw68 in that all of its tryptic peptides could be accounted for in terms of the A2 or Aw68 maps. Here we have isolated genomic clones for both Aw68 and Aw69 and obtained nucleotide sequences for the three major exons and intron 2 from these genes. This has permitted a detailed comparison of the primary structures of A2, Aw68 and Aw69. The results show clearly that HLA-Aw69 is identical in sequence to Aw68 in the α_1 domain and to A2 in the α_2 and α_3 domains. These findings are consistent with the peptide analysis and confirm our hypothesis that Aw69 is a hybrid molecule.

The identities found between the proteins are also true at the level of DNA sequence. The 5' part of the Aw69 gene is identical to Aw68 and the 3' part is identical to A2. Moreover, we found that three different Aw69 genes gave the identical sequence. The results are consistent with the interpretation that the current population of Aw69 genes has arisen from a single recombination event between allelic A2 and Aw68 genes that occurred within the last 330 000 years. The sequences localize the point of recombination to a region of 86 bp flanking the boundary between exon 2 and intron 2.

Previous comparisons of the sequences of class I histocompatibility molecules have shown that relatively short segments of identity are found in regions of polymorphic variation between alleles of different loci (N'Guyen *et al.*, 1985; Ways *et al.*, 1985). This has led to the hypothesis that one mechanism for the diversification of class I genes is gene conversion-like events between *cis*-orientated alleles of different loci. Our analysis of the HLA-Aw69 gene suggests that an additional genetic mechanism may have contributed to HLA-A diversity. A single reciprocal intra-allelic recombination is sufficient to explain our results.

The Aw69 molecule can thus be considered to be the result of an 'exon shuffling' experiment of nature in which the α_1 domain of Aw68 has been combined with the α_2 and α_3 domains of A2. It is of interest that the PA2.1 antibody binds with the same affinity to both A2 and Aw69 molecules (Ways and Parham, 1983). Although the epitope is clearly determined by polymorphic residues of the A2 α_2 domain its structure is not altered by association with the α_1 domain of either A2 or Aw68. This preservation of an A2-derived antigenic determinant in the hybrid molecule probably results from the considerable homology between the parental proteins, A2 and Aw68. This explanation may account for the differences between our results and those of other workers who have reported that *in vitro* 'exon shuffles' between the α_1 and α_2 domains have little antigenic similarity to either parental molecule (Allen *et al.*, 1984; Arnold *et al.*, 1984).

Our conclusion that the PA2.1 antibody binds an epitope composed of residues in the α_2 domain is consistent with the results of Krangel and colleagues. They found that mutations induced *in vitro* in HLA-A2 which altered the A2, Aw69 epitope involved residues between amino acids 97 and 107 or residue 161 (Krangel *et al.*, 1983; Taketani *et al.*, 1983).

We have compared the nucleotide sequence obtained from the Aw68 genomic clones with the protein sequence (Lopez de Castro *et al.*, 1982) and two cDNA sequences (Arnot *et al.*, 1984) which are all derived from material obtained from the LB cell line. A single disagreement was found between the deduced amino acid sequence from the genomic clone and the protein sequence and this serine/glycine difference at position 207 may be attributable to misassignment in the protein sequence. More surprising was the observation that the sequence of the genomic clone does not correspond exactly to either of the cDNA clones studied by Arnot *et al.* (1984). These clones are incomplete and extend from a region close to the 5' end of exon 3 to the end of the coding

sequence. The genomic sequence does not differ from both cDNA clones at any position and is in fact a mosaic of the two cDNA sequences. One explanation for the differences is experimental error, however we believe our sequence to be correct since we sequenced both strands and have checked the region of disagreement very carefully. Other explanations also exist. If the two Aw68 alleles from LB differ by a few nucleotides (as suggested by Arnot *et al.*, 1984) then genetic exchange events involving these two alleles could account for both sets of data. This explanation implies that different sublines of the LB cell line have diverged.

In summary, we have isolated clones encoding the HLA-Aw68 and Aw69 genes and partially sequenced these clones. Comparison of these sequences with the HLA-A2 sequence (Koller and Orr, 1985) shows that HLA-Aw69 was generated by a genetic exchange event involving 5′ sequences of Aw68 and 3′ sequences of HLA-A2. This event may have been a reciprocal intra-allelic recombination suggesting yet another mechanism for the generation of MHC class I polymorphism. Certainly if it involved non-reciprocal genetic exchange (gene conversion) the size of the DNA region exchanged was at least 1 kb and might therefore involve quite different mechanisms to the small genetic exchange events postulated to explain several other MHC polymorphic relationships (Schultze *et al.*, 1983; N'Guyen *et al.*, 1985; Ways *et al.*, 1985).

Materials and methods

Cell lines

Human B-cell lines LB (HLA-Aw68, B40), IDF (HLA-Aw69, A26, B18 and Bw38), BJ (HLA-Aw69, A30, Bw22, B35) and ZM (Aw69, Aw24, B44, B49) were grown in RPMI 1640 supplemented with 2 mM L-glutamine, 0.57 mM pyruvate, 1.14 mM oxaloacetic acid and 10% fetal calf serum. Mouse L cells were maintained in DMEM supplemented as described above. BJ and ZM were HLA typed and kindly provided by Drs P.Antonelli and J.Hansen.

Enzymes and reagents

Restriction endonucleases and T4 DNA ligase were obtained from New England Biolabs. Proteinase K and calf intestinal phosphatase were from Boehringer Mannheim. The large fragment of DNA polymerase I (Klenow) was purchased from Bethesda Laboratories. Radiochemicals were obtained from Amersham.

Construction of partial genomic libraries

Partial genomic libraries were constructed from the cell lines LB, IDF, BJ and ZM as follows. High mol. wt. DNA was extracted from cells after digestion with proteinase K in the presence of SDS. DNA was digested to completion with *Eco*RI and 100–200 μg fractionated on a 38-ml 10–40% sucrose density gradient by centrifugation at 117 000 *g* for 24 h. 0.5-ml fractions were collected and analyzed by agarose gel electrophoresis. A single fraction with fragments of 6–7 kb was selected and after successive ethanol precipitations ligated for 16 h at 12°C with *Eco*RI-cut λ607 DNA. The ligated DNA was packaged *in vitro* by the method of Maniatis *et al.* (1982) and used to infect *Escherichia coli* BNN102 (*hfl* A150). Each library contained $2-5 \times 10^6$ independent recombinants.

The libraries were either screened directly by hybridization or after amplification in BNN102. Approximately 1×10^6 p.f.u. were used to infect *E. coli* c600 and the resulting plaques then screened as described below.

Isolation of HLA-A locus genomic clones

Partial genomic libraries in λ607 were screened by hybridization of replicate filters at high stringency (65°C or 68°C; 0.1 × SSC, 0.1% SDS) with two separate probes. An HLA-A locus-specific probe (pHLA-2a.1; Koller *et al.*, 1984) was generously provided by Dr H.T.Orr (University of Minnesota). An almost full-length human class I MHC cDNA clone (Biro *et al.*, 1983) was the gift of Dr S.M.Weissman (Yale University). Phage plaques hybridizing with both probes were purified to homogeneity. Phage DNA minipreps were performed by the method of Davis *et al.* (1980). DNA prepared in this way was used to confirm the presence of appropriately sized inserts which showed specific hybridization with the HLA-A locus probe, to identify the expressed product of the cloned genes by DNA-mediated gene transfer and to prepare a suitable fragment for subcloning.

DNA-mediated gene transfer

HLA-A locus genomic clones were transfected into mouse fibroblasts (Ltk^- cells) by the calcium phosphate technique (Wigler *et al.*, 1978). Co-selection was achieved by using the herpes simplex thymidine kinase gene to confer HAT resistance. Transfected cells selected by these means were grown as bulk cultures and assayed by cell binding assay. In some cases, individual transfected cells were cloned after assay by limiting dilutions to 0.3 cells per well.

Cell binding assay

Indirect radioimmunoassays for the expression of transfected class I gene products were performed essentially as described (Parham *et al.*, 1982). $2-10 \times 10^5$ cells were incubated with monoclonal antibodies (15 μg/ml) in 50 μl phosphate-buffered saline (PBS) containing 5 mg/ml bovine serum albumin (BSA) (assay buffer) for 1 h at 4°C. Cells were washed three times with assay buffer by centrifugation and then resuspended in 25 μl assay buffer containing 400 000 c.p.m. of affinity-purified $F(ab')_2$ ^{125}I-labelled rabbit anti-mouse IgG (sp. act. $\sim 5 \times 10^7$ c.p.m./μg). Following incubation for 1 h at 4°C the cells were again washed three times and then assayed for bound radioactivity.

Monoclonal antibodies MA2.1 (anti-A2, B17; McMichael *et al.*, 1980), CR11-351 (anti-A2, Aw68, Aw69; Russo *et al.*, 1983), W6/32 (anti-HLA-A, B, C; Barnstable *et al.*, 1978) and 11-4-1 (anti-H-$2K^k$; Oi *et al.*, 1978) were used to define specific HLA products on the surface of transfected mouse cells. In each experiment an A2-expressing human B cell line was used as a positive control. Relevant non-transfected mouse cells served as negative controls in every case.

DNA sequencing

Sequencing was performed by the dideoxy method of Sanger *et al.* (1977). A 5.1-kb *Hind*III fragment containing the complete HLA-A gene was subcloned into M13mp19 and recombinant clones isolated for both orientations. Selected regions of the gene were sequenced by means of specific oligonucleotide primers or by using the M13 universal 17-bp primer (P-L Biochemicals).

Oligonucleotide primers were designed by inspection of the nucleic acid sequences for HLA-A2 (Koller and Orr, 1985), A3 (Strachan *et al.*, 1984), B7 (S.M.Weissman, personal communication), CW3 (Sodoyer *et al.*, 1984) and pHLA-12.4 (Malissen *et al.*, 1982). Primer consensus sequences were selected for optimal matching on all five prototype templates using the following criteria: (i) there were no non-specific sites of mismatch with a T_m (calculated by an empirical formula; Suggs *et al.*, 1981) < 11°C below the specific site T_m. (ii) The T_m at the specific site was at least 56°C. (iii) The 3′ end of the oligonucleotides was at least 15 nucleotides 5′ of the start of the region to be sequenced. In total, eight oligonucleotide primers were designed and these permitted sequencing of exons 2, 3 and 4 and of intron 2 on both strands. These primers were 18–26 bases in length and had 0–3 mismatches with the prototype template DNAs.

Oligonucleotide primers were synthesized using an Applied Biosystems 380A DNA synthesizer (kindly performed by Courtney Moulds). Full-length products were purified by electrophoresis in 20% acrylamide-urea gels. Primers were hybridized with appropriate template DNA at 68°C in 20 mM Tris pH 8.5, 5 mM $MgCl_2$ under empirically determined conditions of template excess. After incubation for 1 h the mixtures were allowed to cool slowly to room temperature and used as substrates for standard dideoxy sequencing reactions using [^{35}S]dATP as label (Biggin *et al.*, 1983).

Acknowledgements

We thank Harry Orr and Sherman Weissman for gifts of DNA probes and for sharing data prior to publication; Paolo Antonelli for the BJ and ZM cell lines; Helene Coppin for vector DNA and assistance with transfections; and Anne McIntyre for typing the manuscript. This work was supported by grants from the US Public Health Service NIH AI17892 and the Searle Scholars program of the Chicago Community Trust. N.H. was supported by a post-doctoral fellowship from the Muscular Dystrophy Association.

References

Albert,E.D., Baur,M.P. and Mayr,W.R., eds. (1984) *Histocompatibility Testing 1984*, published by Springer-Verlag, Berlin, FRG.

Allen,H., Wraith,D., Pala,P., Askonas,B. and Flavell,R.A. (1984) *Nature*, **309**, 279-281.

Antonelli,P., Choo,S.Y., Nisperos,B. and Hansen,J.A. (1985) *Tissue Antigens*, in press.

Arnold,B., Burgert,H-G., Hamann,U., Hammerling,G., Kees,U. and Kvist,S. (1984) *Cell*, **38**, 79-84.

Arnot,D., Lillie,J.W., Auffray,C., Kappes,D. and Strominger,J.L. (1984) *Immunogenetics*, **20**, 237-252.

Barnstable,C.J., Bodmer,W.F., Brown,G., Galfre,C., Milstein,C., William,A.F. and Ziegler,A. (1978) *Cell*, **14**, 9-20.

Biddison,W.E., Kostyu,D.D., Strominger,J.L. and Krangel,M.S. (1982) *J. Immunol.*, **129**, 730-734.
Biggin,M.D., Gibson,T.J. and Hong,G.F. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 3963-3965.
Biro,P.A., Pan,J., Sood,A.K., Kole,R., Reddy,V.B. and Weissman,S.M. (1983) *Cold Spring Harbor Symp. Quant. Biol.*, **47**, 1079-1086.
Brodsky,F.M., Parham,P., Barnstable,C.J., Crumpton,M.J. and Bodmer,W.F. (1979) *Immunol. Rev.*, **47**, 3-61.
Davis,R.W., Botstein,D. and Roth,J.R. (1980) *Advanced Bacterial Genetics*, published by Cold Spring Harbor Laboratory Press, NY.
Gotch,F.M., Kelly,C., Ellis,S.A., Wallace,L., Rickinson,A.B., Van der Poel,J., Crumpton,M.J. and McMichael,A.J. (1985) *Immunogenetics*, **21**, 11-24.
Hayashida,H. and Miyata,T. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 2671-2675.
Holmes,N. and Parham,P. (1984) *Immunogenetics*, **20**, 103-115.
Kimball,E.S. and Coligan,J.E. (1983) *Contemp. Top. Mol. Immunol.*, **9**, 1-63.
Koller,B.H. and Orr,H.T. (1985) *J. Immunol.*, **134**, 2727-2733.
Koller,B.H., Sidwell,B., DeMars,R. and Orr,H.T. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 5175-5178.
Krangel,M.S., Taketani,S., Pious,D. and Strominger,J.L. (1983) *J. Exp. Med.*, **157**, 324-336.
Lopez de Castro,J.A., Strominger,J.L., Strong,D.M. and Orr,H.T. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 3813-3817.
McMichael,A.J., Parham,P., Rust,N. and Brodsky,F. (1980) *Hum. Immunol.*, **1**, 121-129.
Malissen,M., Malissen,B. and Jordan,B.R. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 893-897.
Maniatis,T., Fritsch,E.F. and Sambrook,J. (1982) *Molecular Cloning. A Laboratory Manual*, published by Cold Spring Harbor Laboratory Press, NY.
N'Guyen,C., Sodoyer,R., Trucy,J., Strachen,T. and Jordan,B.R. (1985) *Immunogenetics*, **21**, 479-490.
Oi,V.T., Jones,P.P., Goding,J.W., Herzenberg,L.A. and Herzenberg,L.A. (1978) *Curr. Top. Microbiol. Immunol.*, **81**, 115-129.
Parham,P. and Brodsky,F.M. (1981) *Hum. Immunol.*, **3**, 277-299.
Parham,P., Androlewicz,M.J., Brodsky,F.M., Holmes,N.J. and Ways,J.P. (1982) *J. Immunol. Methods*, **53**, 133-173.
Perler,F., Efstratiadis,A., Lomedico,P., Gilbert,W., Kolodner,R. and Dodgson,J. (1980) *Cell*, **20**, 555-566.
Ploegh,H.L., Orr,H.T. and Strominger,J.L. (1981) *Cell*, **24**, 287-299.
Russo,C., Ng,A.K., Pellegrino,M.A. and Ferrone,S. (1983) *Immunogenetics*, **18**, 23-35.
Sanger,F., Nicklen,S. and Coulson,A.R. (1977) *Proc. Natl. Acad. Sci. USA*, **74**, 5463-5467.
Schultze,D.H., Pease,L.R., Geier,S.S., Reyes,A.A., Sarmiento,L.A., Wallace, R.B. and Nathenson,S.G. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 2007-2011.
Sodoyer,R., Damotte,M., Delovitch,T., Trucy,J., Jordan,B.R. and Strachan,T. (1984) *EMBO J.*, **3**, 879-885.
Strachan,T., Sodoyer,R., Damotte,M. and Jordan,B.R. (1984) *EMBO J.*, **3**, 887-894.
Suggs,S.V., Hirose,T., Miyake,T., Kawashima,E.H., Johnson,M.J., Itakura,K. and Wallace,R.B. (1981) *ICN-UCLA Symp. Mol. Cell. Biol.*, **23**, 683-693.
Taketani,S., Krangel,M.S., Pious,D. and Strominger,J.L. (1983) *J. Immunol.*, **131**, 2935-2938.
Van der Poel,J.J., Pool,J., Goulmy,E. and van Rood,J.J. (1983) *Immunogenetics*, **17**, 599-608.
Ways,J.P. and Parham,P. (1983) *Biochem. J.*, **216**, 423-432.
Ways,J.P., Coppin,H.L. and Parham,P. (1985) *J. Biol. Chem.*, in press.
Wigler,M., Pellicer,A., Silverstein,S. and Axel,R. (1978) *Cell*, **14**, 725-731.

Received on 24 July 1985