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Molecular analysis of HLA-A2.4 functional variant KLO: close structural and evolutionary relatedness to the HLA-A2.2 subtype

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Abstract. The structure of an HLA A2 4 functional variant (A2 4c) expressed on donor KLO has been examined by comparative peptide mapping with other HLA A2 antigens of known structure and radiochemical sequencing All the peptide differences between A2 4c and A2 1 could be accounted for by five amino acid changes at positions 9, 43, 66, 95, and 156 The nature of residues 9, 43, and 95 in A2 4c was determined by sequencing to be identical to those in A2 2Y The nature of residue 156 in A2 4c was also assigned as identical to that in A2 2Y on the basis of the identity of the corresponding peptide in its chromatographic comparison with A2 2Y Position 66 was unique to A2 4c It was determined to be an Asn residue instead of the Lys present in all other HLA-A2 antigens of known structure This was the only detected amino acid difference between A2 4c and A2 2Y The results indicate that, from a structural point of view, A2 4c is most closely related to the A2 2 subtype antigens and not to other A2 4 antigens The data are compatible with the assumption that A2 4c was derived from A2 2Y by a single point mutation event

Introduction

Class I HLA antigens which appear to be virtually homogeneous by tissue typing serologic reagents are, in a number of cases, amenable to further subdivision by cytolytic T lymphocytes (CTL), isoelectric focusing (IEF), or other criteria The molecular analysis of this heterogeneity provides a basis to outline the pathways of HLA diversification and to examine the influence of limited structural changes in modulating the specificity of CTL recognition Both of these aspects are particularly well illustrated in the case of HLA-A2 Since the initial observation that influenza-virus-specific, HLA-A2 restricted CTL failed to kill some virus-infected A2 target cells (Bidison et al 1980), numerous studies have contributed to establish that HLA-A2 is a family of functionally and biochemically distinguishable proteins Comprehensive studies using alloreactive CTL populations and IEF defined four subtypes designated A2 1, A2 2, A2 3, and A2 4 (van der Poel et al 1983) These subtypes were also partially distinguished by HLA A2-restricted CTL in a variety of antigenic systems such as influenza and Epstein-Barr viruses and minor H-Y and HA histocompatibility antigens (Biddison et al 1982, Gaston et al 1983, Goulmy et al 1984) Further heterogeneity within some of these subtypes has been detected with both alloimmune and self-restricted antigen-specific CTL, as well as with monoclonal antibodies (Gotch et al 1985, van der Poel et al 1986, Kennedy et al 1987)

The structures of HLA-A2 1 (Koller and Orr 1985), the two known variants of A2 2 (A2 2F and A2 2Y), and A2 3 have been determined A2 2F differs from A2 1 by three amino acid replacements at positions 43, 95, and 156, A2 2Y having an additional change at position 9 (Mattson et al 1987, Holmes et al 1987) A2 3 and A2 1 differ in three clustered changes at positions 149, 152, and 156 (Krangel et al 1983, Mattson et al 1987) A rare variant, OZB, defined functionally as A2 1 but having a behavior in IEF-like A2 3 (van der Poel et al 1986), was shown to differ from A2 1 by a single amino acid change at position 236 in the $\alpha 3$ domain (Castaño et al 1988) The HLA-A2 4 subtype includes several variants that are distinguishable from A2 1 and among themselves by CTL, but not by IEF (van der Poel et al 1986) The structure of two A2 4 antigens, expressed on CLA (A2 4a) and KNE cells (A2 4b), has been analyzed by peptide mapping and protein sequencing, and each was found to differ from A2 1 in a single amino acid residue at position 9 (Ezquerra et al 1986) or 99 (Domenech et al 1988), respectively

Here we report on the structure of a third A2 4 variant KLO (hereon referred to as A2 4c) Unlike A2 4a and A2 4b, A2 4c is structurally closer to the A2 2 subtype,

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differing from A2 1 in five amino acid sequence positions and from A2 2Y by one single amino acid change

Materials and methods

Purification of radiochemically labeled HLA A2 heavy chains The lymphoblastoid cell lines JY (HLA A2 1 B7) W149 (HLA A2 2Y Bw58) and KLO (HLA A1 A2 4c B8 Bw50) were used as the source of material The radiochemical purification of all A2 heavy chains was performed by immunoprecipitation with the A2/Aw69 specific monoclonal antibody PA2 1 (Parham and Bodmer 1978) and antidenatured HLA A B heavy chain serum as described (Fzquerra et al 1986)

Peptide mapping and sequencing The structural analysis of HLA A2 4c by comparative peptide mapping and radiochemical sequencing was as described (Fzquerra et al 1986) Tryptic maps of jointly digested A2 1 or A2 2Y and A2 4c heavy chains were obtained by high performance liquid chromatography (HPLC) in a gradient of ammonium acetate and acetonitrile as described in detail elsewhere (Vega et al 1985) Further fractionation of some unresolved peptide mixtures was carried out in HPLC with a gradient of acetonitrile and trifluoracetic acid following a described procedure (Rojo et al 1987)

Results

A2 4c is structurally related to the A2 2 subtype The initial strategy used for the structural characterization of A2 4c involved its biochemical comparison with A2 1 by double-label tryptic mapping of Lys- and Arg labeled peptides These two amino acids label all tryptic peptides from both molecules except the carboxyl-terminal ones The result of such comparison is shown in Figure 1 The Lys-labeled map showed two ^{14}C-labeled difference peptides, K2 and K7, and a ^{3}H labeled difference peptide, K3 The Arg-labeled map displayed a more complex pattern of differences, with five ^{14}C-labeled (R8, R12, R28, R29, and R32) and five ^{3}H-labeled (R7, R9, R13, R30, and R31) difference peaks R30 was a mixture of two ^{3}H-labeled difference peptides as assessed by rechromatography of this peak material under different chromatographic conditions (not shown) Sequencing of the A2 4c peptides R7 and R9 yielded ^{3}H-radioactivity at cycles 8 and 1, respectively (Fig 1C) As will be shown below, these data indicate that R7 and R9 are the peptides spanning residues 36–43 (FDSDAASR) and 44–48 (RMEPR), respectively, and that Gln_{43} in A2 1 was changed to Arg_{43} in A2 4c

To identify the nature of the difference peptides shown in Figure 1, Ala-, Trp-, Leu-, and Tyr labeled maps were obtained The Ala-labeled map (Fig 2A) showed three ^{14}C labeled (A3, A5, and A15) and three ^{3}H-labeled (A2, A6, and A16) difference peptides They eluted at the same positions as R8, R12, R28, R7, R13, and R30, respectively The sequence analyses of the Ala-labeled difference peptides are shown in Figure 2B A2 and A3 both have radioactivity at cycles 5 and 6, indicating that they are the A2 4c and A2 1 counterparts, respectively, of the peptide spanning residues 36–43 (in A2 4c) or 36–44 (in A2 1) A5 and A6 each have radioactivity at position 8 The only tryptic peptide from A2 1 with Ala at this position is the glycopeptide, spanning residues 83–97 (GYYNQSEAGSHTVQR) Thus, A5 and A6 are the A2 1 and the A2 4c counterparts of this peptide, respectively A15 was shown upon sequencing to have ^{14}C-radioactivity at cycles 5, 6, and 9, consistent with this peptide being the one spanning residues 145–157 from A2 1 (HKWEAAHVAEQLR) A16 was recovered with very low yield and could not be sequenced From its elution position (see below) it was assumed to be the ^{3}H-labeled counterpart for A15 The low yield of this peptide is a consequence of the partial tryptic cleavage at Lys_{146} The Trp labeled map (Fig 2C) was identical except for the presence of the A2 1 difference peak W9, eluting as A15 and R28, and the A2 4c difference peak W10 eluting as A16 and R30 Sequencing of W9 and W10 showed radioactivity at cycle 3 in both of them, confirming their assignment as the peptides spanning residues 145–157 in A2 1 and A2 4c, respectively The only other tryptic peptide from A2 1 with Trp at this position, the one spanning residues 49–65, eluted with a much shorter retention time (unpublished observations) In the Leu-labeled map (Fig 2D) a single ^{3}H-labeled difference peptide, L5, was apparent, eluting as R13 and A6 Sequencing of this peptide showed radioactivity at cycle 13 (Fig 2D) This result, together with the elution position of L5 and the absence of a ^{14}C-labeled counterpart, indicated that it was the glycopeptide from A2 4c, and that this molecule possessed Leu at position 95 instead of the Val-residue present in A2 1 In addition, a ^{14}C-difference peptide was found, co-eluting with an identical peptide, upon rechromatography of L15 under different chromatographic conditions (not shown) Because of its low yield, this peptide could not be sequenced, but it was assumed to be on the basis of its elution position, the A2 1 peptide spanning residues 145–157 No ^{3}H-labeled counterpart for this peptide was found The Tyr-labeled map showed two ^{14}C labeled (Y5 and Y14) and two ^{3}H-labeled (Y6 and Y16) difference peaks (Fig 3A) Y5 and Y6 eluted in the same positions as R12 or A5 and R13, A6 or L5, respectively Sequencing of these two peptides yielded radioactivity at cycles 2 and 3 in both of them (Fig 3B) These results confirm that Y5 and Y6 are the A2 1 and A2 4c glycopeptides, respectively Y14, which eluted as R29 was shown upon sequencing to have radioactivity at cycle 1 This peptide was the one spanning residues 7–14 or 7–17 from A2 1 (YFFTSVSRPGR) because this is the only tryptic peptide with Tyr at position 1 lacking Lys, and no difference peptides were found eluting late in the Lys-labeled map (Fig 1) Y16, which eluted as R30, showed radioactivity at cycles 1 and 3, indicating that this was an A2 4c peptide spanning residues 7–14 or 7–17, and the Phe_9 in A2 1 was changed to Tyr_9 in A2 4c Equivalent peptides

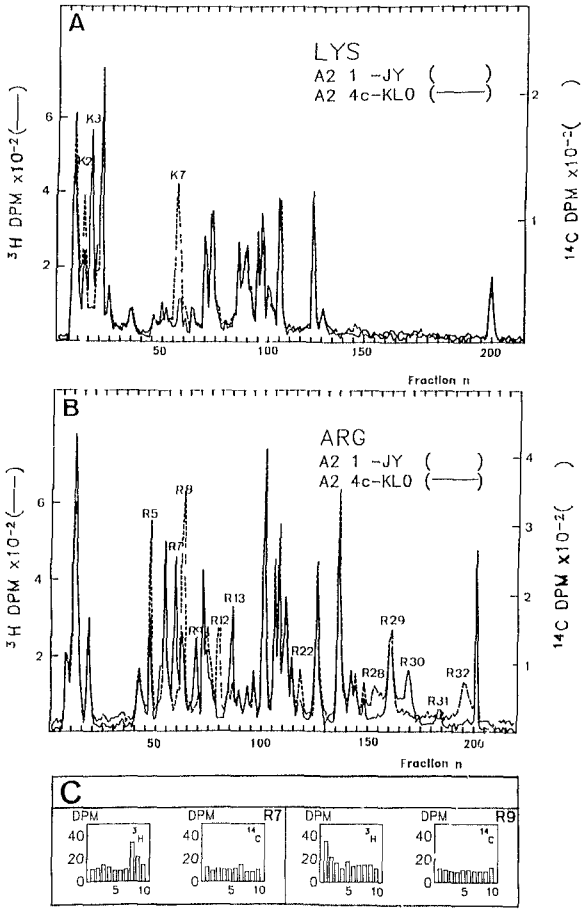
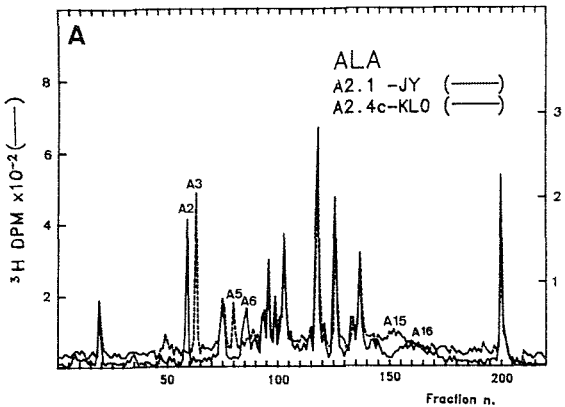
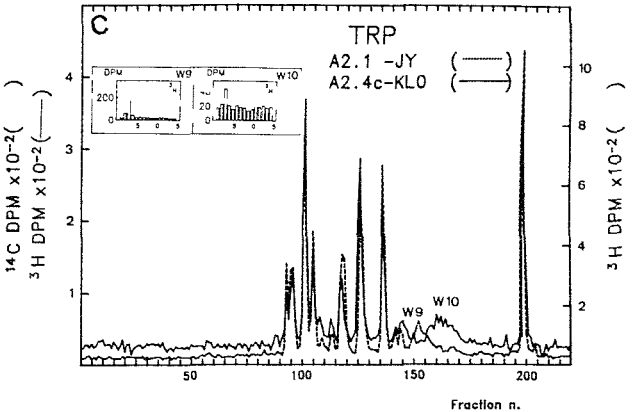


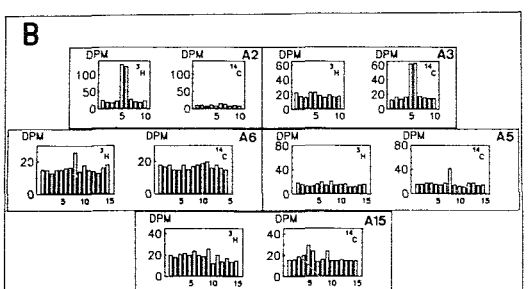
Fig. 1 **A** and **B** show the reverse phase HPLC comparison of the Lys labeled and Arg labeled tryptic peptides respectively between A2 1 (JY) and A2 4c (KLO) Peaks are numbered according to their relative elution position within each peptide map Peaks referred to in the text are specified R5 and R22 peaks were not difference peptides as assessed by rechromatography of each peak under different conditions (see Materials *and methods*) *Rechromatography of R29* revealed the presence of a ^{14}C difference peptide co eluting in this peak with an identical peptide (see text) Rechromatography of R30 showed the presence of two ^{3}H labeled difference peptides **C** shows the radiochemical sequence analyses of R7 and R9 For each peptide ^{3}H(A2 4c) and ^{14}C(A2 1) radioactivity (y axis) is separately plotted against the cycle number (x axis) in the left and right histogram respectively

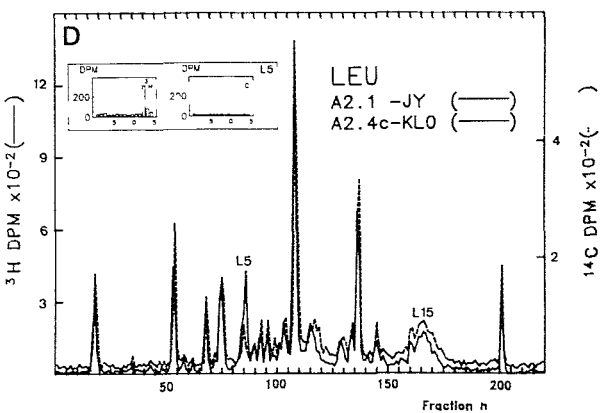
for R31 and R32 were not found However, these two peptides are probably related to R30 and R29, respectively, as a result of partial tryptic cleavage at the Arg_{14}-Pro_{15} bond, as was previously observed (Ezquerra et al 1986) Cleavage at this bond may vary between experiments, affecting the yield of the corresponding peptides

The results from the comparative analysis of A2 4c with A2 1 described above are summarized in Figure 4 Taken together, they indicate the following 1) A2 4c has changes in the tryptic peptides including all positions in which A2 2Y and A2 1 are different, namely, residues 9, 43, 95, and 156 These peptides accounted for all the difference peaks detected in the Arg-, Ala-, Trp-, Leu-, and Tyr-labeled maps 2) The changes between A2 1 and A2 4c at positions 9, 43, and 95 are the same as those present in A2 2Y The existence of a change at position 156 was not directly confirmed by sequencing 3) The Lys labeled difference peptides were not related to those


A
ALA
A2.1 -JY
A2.4c-KLO
A2
A3
A5
A6
A15
A16
3H DPM x10-2
Fraction n.


C
TRP
A2.1 -JY
A2.4c-KLO
W9
W10
14C DPM x10-2
3H DPM x10-2
Fraction n.


B
DPM
A2
A3
A6
A5
A15


D
LEU
A2.1 -JY
A2.4c-KLO
L5
L15
3H DPM x10-2
14C DPM x10-2
Fraction n

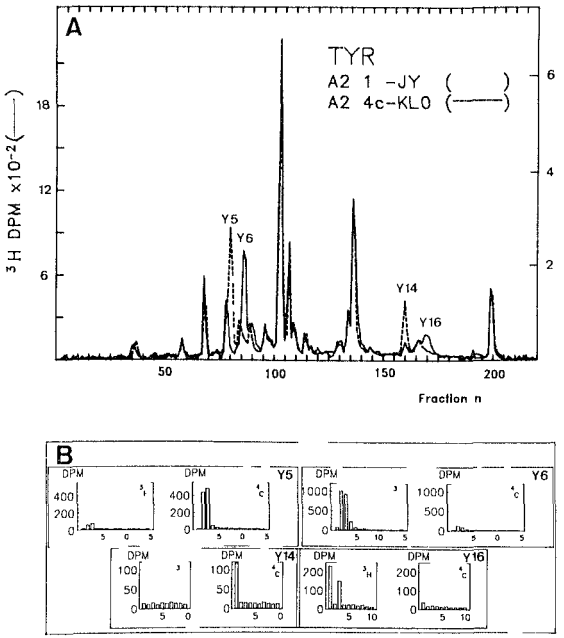


Fig 3 A shows the reverse phase HPLC comparison of the Tyr labeled tryptic peptides between A2 1 and A2 4c B shows the sequence analyses of the Tyr labeled difference peptides Numbering and sequencing of peptides is as in Figure 1

analyzed in the other peptide maps suggesting that A2 4c has one or more substitutions in addition to those present in the A2 2Y subtype

A2 4c and A2 2Y differ by a single amino acid change at position 66 In a second set of experiments Arg and Lys labeled comparative peptide maps between A2 2Y and A2 4c were obtained The Arg labeled map was identical (Fig 5A) The Lys labeled map showed two ^{14}C labeled (K1 and K4) difference peaks and one ^{3}H labeled (K2) difference peak (Fig 5B) All three difference peptides eluted in the same positions as those in the comparative Lys labeled map with A2 1 (Fig 1A) suggesting that they were the same peptides The two ^{14}C-difference peptides K1 and K4 showed radioactivity at cycle 2 and at cycles 1 and 3 respectively (Fig 5B) This result was compatible with K1 and K4 being the peptides spanning residues 67–68 (VK) and 66–68 (KVK), respectively They would have resulted from partial tryptic cleavage at the Arg_{65} and Lys_{66} residues in A2 2Y The possibility that K4 was a longer peptide spanning residues 66–75 (KVKAHSQTHR) was ruled out because the His labeled map was identical (not shown) Sequencing of the A2 4c peptide K2 yielded radioactivity only at cycle 3 This suggested that K2 was the 66–68 peptide, and that Lys_{66} could have changed in A2 4c The Val labeled map (Fig 6A) showed only a ^{14}C labeled (V2) and a ^{3}H labeled (V3) difference peptide eluting in the same positions as K1 and K2 respectively As expected, sequencing of V2 and V3 yielded radioactivity at cycle 1 and at cycle 2 respectively (Fig 6A) This result confirmed the assignment of the two difference peptides as those spanning residues 67–68 in A2 2Y and 66–68 in A2 4c In addition, it indicated that Val_{67} was not changed in A2 4c In the Val labeled map

◀ Fig 2 A shows the reverse phase HPLC comparison of the Ala labeled tryptic peptides between A2 1 and A2 4c B shows the sequence analyses of the Ala labeled difference peptides C and D show the reverse phase HPLC comparison of the Trp labeled and Leu labeled tryptic peptides respectively between A2 1 and A2 4c For the comparison of Trp labeled peptides ^{3}H labeled maps from A2 1 and A2 4c were separately obtained and were plotted together The sequence analyses of the corresponding difference peptides are included in each map 1 5 was purified by rechromatography under different conditions prior to sequencing The 1 15 peak included a ^{14}C difference peptide coeluting with an identical one (see text) Numbering and sequencing of peptides is as in Figure 1

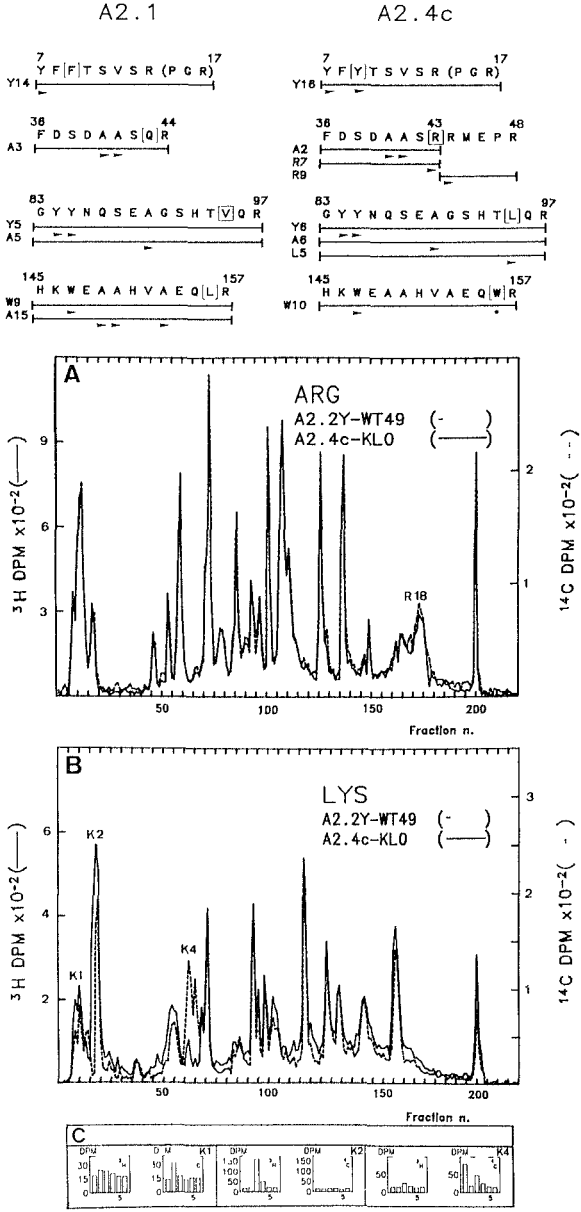


Fig. 4. Summary of the amino acid sequence analyses of the HLA A2 difference peptides from A2 1 (JY) and A2 4c (KLO) The assignment of each single-labeled difference peptide used for sequencing is shown The assignments of other difference peptides which were not sequenced and were identified on the basis of their elution position are described in the text These include A16 I 15, the I ys labeled and most of the Arg labeled peptides *Arrows* denote residues identified directly by radiochemical sequencing The *asterisk* under Trp_{156} from the W10 peptide indicates that this residue was not directly sequenced (see text) Amino acid differences between both molecules are *boxed* The standard one letter code for amino acids is used

Fig. 5. **A** and **B** show the reverse phase HPLC comparison of Arg labeled and I ys labeled tryptic peptides respectively, from A2 2Y (WT49) and A2 4c (KLO) R18 was shown by rechromatography under different conditions to consist of a mixture of two *identical peptides* **C** shows the sequence analyses of the Lys labeled difference peptides All three K1, K2 and K4 peptides were purified from contaminant identical peptides by rechromatography under different conditions prior to sequencing Numbering and sequencing of peptides is as in Figure 1

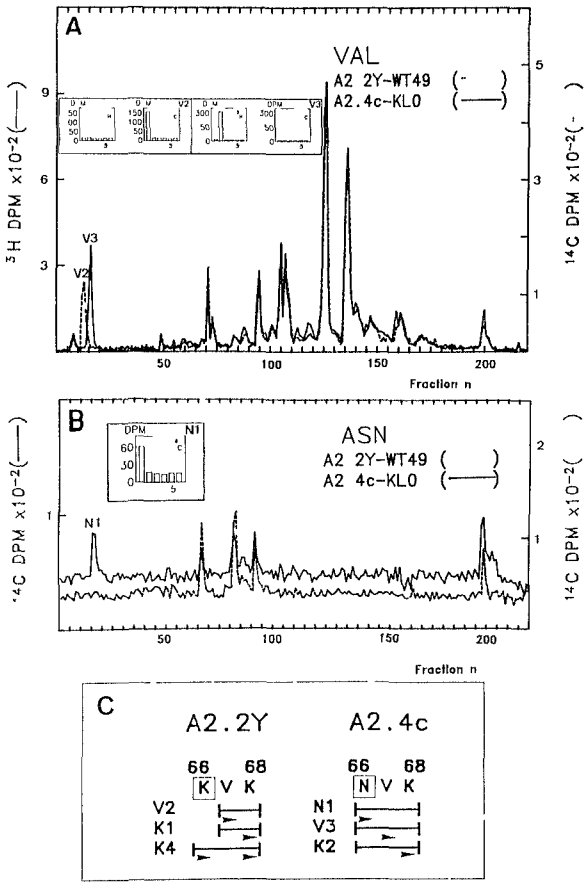


Fig 6 **A** and **B** show the reverse phase HPLC comparison of Val labeled and Asn labeled tryptic peptides respectively from A2 2Y and A2 4c For the comparison of the Asn labeled peptides ^{14}C labeled maps from both molecules were separately obtained and were plotted together The sequence analyses of the corresponding difference peptides are included in each map Numbering and sequencing of peptides is as in Figure 1 **C** is a summary of the assignment and amino acid sequencing of the detected difference peptides between A2 4c and A2 2Y *Arrows* denote residues identified directly by radiochemical sequencing The single detected difference between both molecules is *boxed*

an equivalent to the partial cleavage product K4 was not observed The nature of the change at position 66 was established by obtaining the Asn-labeled map (Fig 6B) This amino acid was chosen because it is frequently found at this position in class I HLA antigens The only difference peak found was the ^{3}H-labeled N1, whose elution position was the same as that of K2 and V3 Its sequencing showed radioactivity at cycle 1 (Fig 6B), confirming that Lys_{66} in A2 2Y was changed to Asn_{66} in A2 4c The sequencing and assignment of the difference peptides found in the comparison of A2 2Y and A2 4c is summarized in Figure 6C

Discussion

The strategy used for the structural characterization of the HLA-A2 4c antigen variant expressed on KLO cells was based on the biochemical comparison of all tryptic peptides of the molecule with those of two other HLA-A2 antigens of known structure HLA-A2 1 and HLA-A2 2Y The reliability of this approach has been discussed elsewhere (Vega et al 1986, Domenech et al 1988) Comparative analysis with HLA-A2 1 revealed that A2 4c was very similar to A2 2Y This antigen differs from A2 1 at positions 9, 43, 95, and 156 (Holmes et al 1987) The

four changes were also present in A2 4c This was established for residues 9 43, and 95 by direct sequencing The nature of the change at position 156 could not be determined by sequencing but it was assigned on the following bases

a) the existence of a difference peptide spanning residues 145–157 in the comparison of A2 4c with A2 1 This peptide was labeled in both A2 1 and A2 4c with Arg (one of the two peptides in R30) Ala (A16) and Trp (W10) but was only recovered from A2 1 (the difference peak within L15) and not from A2 4c upon Leu-labeling,

b) the identity of the Arg labeled map in the comparison of A2 4c with A2 2Y

Furthermore this identity strongly suggests that there are no additional substitutions within the Arg labeled difference peptides detected in the comparison of A2 4c with A2 1 (Fig 4)

The only peptide differences found between A2 4c and A2 1 which were not accounted for by the changes present in A2 2Y were those in the Lys-labeled map (Fig 1A) As expected these peptides were also found in the comparison between A2 4c and A2 2Y (Fig 5) and shown to reflect a single change of Lys_{66} in A2 1 and A2 2Y to Asn_{66} in A2 4c Thus within the limitations of the peptide mapping analysis our results indicate that A2 4c differs from A2 1 at positions 9, 43 66, 95 and 156 and from A2 2Y only by the change at position 66

This substitution which requires a single base change at the DNA level, accounts for the IEF pattern of A2 4c which is identical with that of A2 1 and is one charge unit more acidic than A2 2 (van der Poel et al 1983) It illustrates that subtype classifications of class I antigens based on IEF do not necessarily reflect distinct subtype related structural patterns Indeed, from a structural viewpoint, A2 4c would be a new variant of the A2 2 subtype Figure 7 shows a comparison of all HLA A2 antigens of known structure Three groups can clearly be distinguished on the basis of their structural patterns A first group includes A2 2F A2 2Y and A2 4c, all of which as mentioned above differ from A2 1 by the same changes at positions 43, 95, and 156 A2 2Y and A2 4c both have the same additional change at position 9, A2 4c having yet another change at position 66 A second group consists of A2 3, which differs from A2 1 by three clustered changes in the $\alpha 2$ domain A third group includes A2 4a, A2 4b, and the A2 1/A2 3 variant OZB each one differing from A2 1 by one amino acid change located in the $\alpha 1$, $\alpha 2$ or $\alpha 3$ domains, respectively

The evolutionary relationships within the HLA A2 antigen family are uncertain It has been suggested that *A2 1* and *A2 2Y* are relatively distant alleles whose divergence probably involved multiple point mutations and a genetic exchange event Reciprocal recombination between these two alleles might have originated A2 2F and A2 4a (Holmes et al 1987) The origin of A2 3 is most easily explained by a single gene conversion like event from A2 1 (Mattson et al 1987, Holmes et al 1987) In addition, single point mutations from A2 1 could have generated A2 4b and OZB (Domenech et al 1988 Castaño et al 1988), and a point mutation from A2 2Y could have given rise to A2 4c These putative diversification pathways are summarized in Figure 8 However it should be emphasized that there are no systematic studies on the racial distribution of the various HLA A2 subtypes although A2 1 is the predominant subtype and A2 3 has only been found in Orientals (van der Poel et al 1983) Furthermore, the ancestral allele of the A2 family potential donor sequences for the putative nonreciprocal recombination events, and possible intermediate forms have not been identified In addition complete DNA sequences are necessary for all members of the family to substantiate postulated point mutations In the absence of this information, alternative evolutionary pathways cannot be ruled out For instance, it is also possible that A2 4a and A2 2F

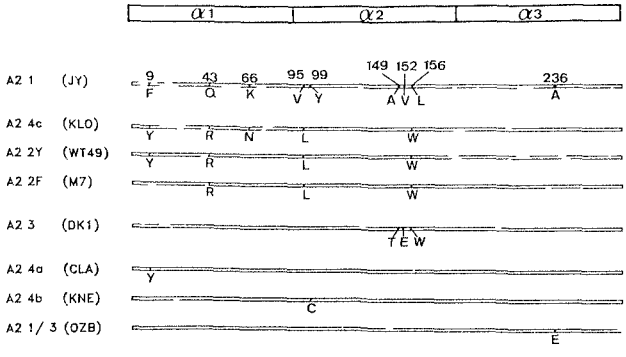


Fig 7 Location of amino acid substitutions among HLA A2 natural variants showing the structural relationship of A2 4c to the A2 2 subtype antigens Those positions in which variants are different from A2 1 are specified by number The corresponding residues are identified with the one letter code for amino acids References are given in the text

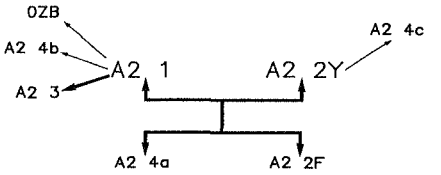


Fig 8 A hypothetical scheme showing the evolutionary diversification of HLA A2 antigens *Thick arrows* indicate reciprocal or nonreciprocal DNA exchange events *Thin arrows* denote point mutation events

were intermediate forms in a pathway going from A2 1 to A2 2Y Other alternatives have been discussed previously (Holmes et al 1987)

Position 66 which distinguishes A2 4c from A2 2Y is located in the long α helix from the α1 domain which is a part of the antigen binding and T cell recognition site of HLA A2 (Borjkman et al 1987) It is spatially close to positions 9 and 95 which as mentioned above are also changed in A2 4c and A2 2Y with respect to A2 1 Since residue 66 is pointing towards the site it would not be expected to be conspicuous for antibody recognition but it could be important for T cell recognition

The A2+B17 specific monoclonal antibody MA2 1 (McMichael et al 1980) is thought to recognize an epitope involving residues 62–65 (Ways et al 1985) The introduction of a single change of Lys to Ile at position 66 in A2 1 through site directed mutagenesis was sufficient to completely abrogate recognition by this antibody suggesting that its determinant could also include residue 66 (Santos Aguado et al 1988a) The reactivity of MA2 1 with KLO cells was examined by flow cytometry analysis and found to be indistinguishable to that obtained with other A2 specific antibodies such as PA2 1 or CR11 351 (Russo et al 1983) (data not shown) Thus HLA A2 4c expresses the antigenic determinant recognized by MA2 1 This suggests that the change of Lys_{66} to Asn_{66} present in A2 4c does not affect recognition by this antibody It appears that the nature of residue 66 influences the epitope recognized by MA2 1 so that strongly nonconservative substitutions (such as Ile) at this position may interfere with the binding of this antibody while other changes (such as Asn) will not

With regard to the cellular recognition HLA A2 restricted H Y specific CTL do not recognize HLA A2 4c cells (Goulmy et al 1984 A2 4c is on target cell no 1 in Table 1 from the latter reference) This absence of recognition cannot unambiguously be attributed to the change at position 66 because other A2 2 target cells which share with A2 4c several substitutions at other positions also failed to be recognized by these CTLs (Goulmy et al 1984) The participation of position 66 in allogeneic T cell recognition is suggested from the fact that polyclonal CTL raised against A2 2 antigens in a variety of responder individuals consistently showed diminished reactivity for A2 4^+ target cells including the A2 4c cells In addition a polyclonal CTL line raised from A2 responder cells stimulated with A2 $4a^+$ cells could distinguish between A2 2 and A2 4c target cells (van der Pool et al 1986) However in these studies it was not established whether A2 2F alone or both A2 2F and A2 2Y were represented in the target cell panel In the former situation the molecular interpretation of the reaction patterns in terms of the role of position 66 would be ambiguous because A2 2F and A2 4c also differ at position 9 More detailed studies concerning the ability of T cells to distinguish between A2 2 and A2 4c antigens are presently in progress (E Goulmy personal communication) Another approach to demonstrate the importance of residue 66 in allogeneic T cell recognition was the screening of the reactivity patterns of a set of A2 specific CTL clones against a panel of transfected target cells expressing various site directed mutants It was found that substitution of Lys_{66} in A2 1 by Ile was sufficient to abrogate recognition of the corresponding transfected target cells by six of nine clones amenable to analysis (Santos Aguado et al 1988b)

The structural dissection of the HLA A2 antigen family may now be used as a basis for more discriminative CTL studies aimed at establishing the functional significance of subtype related polymorphism within HLA A2 The structural differences among subtypes often determine significant differences in CTL recognition although many of these variants have not yet been distinguished by antibodies This is of great functional significance because it implies that the immunological specificity of class I MHC antigens may be efficiently modulated through limited structural diversification which can be attained by one or very few genetic events

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