

Identifying Human MHC Supertypes Using Bioinformatic Methods

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Classification of MHC molecules into supertypes in terms of peptide-binding specificities is an important issue, with direct implications for the development of epitope-based vaccines with wide population coverage. In view of extremely high MHC polymorphism (948 class I and 633 class II HLA alleles) the experimental solution of this task is presently impossible. In this study, we describe a bioinformatics strategy for classifying MHC molecules into supertypes using information drawn solely from three-dimensional protein structure. Two chemometric techniques—hierarchical clustering and principal component analysis—were used independently on a set of 783 HLA class I molecules to identify supertypes based on structural similarities and molecular interaction fields calculated for the peptide binding site. Eight supertypes were defined: A2, A3, A24, B7, B27, B44, C1, and C4. The two techniques gave 77% consensus, i.e., 605 HLA class I alleles were classified in the same supertype by both methods. The proposed strategy allowed “supertype fingerprints” to be identified. Thus, the A2 supertype fingerprint is Tyr⁹/Phe⁹, Arg⁹⁷, and His¹¹⁴ or Tyr¹¹⁶; the A3-Tyr⁹/Phe⁹/Ser⁹, Ile⁹⁷/Met⁹⁷ and Glu¹¹⁴ or Asp¹¹⁶; the A24-Ser⁹ and Met⁹⁷; the B7-Asn⁶³ and Leu⁸¹; the B27-Glu⁶³ and Leu⁸¹; for B44-Ala⁸¹; the C1-Ser⁷⁷; and the C4-Asn⁷⁷. *The Journal of Immunology*, 2004, 172: 4314–4323.

Molecules of the MHC bind small peptides and present them on the surface of cells for recognition by TCR-bearing T cells. Formation of the ternary complex between TCR and the peptide-MHC complex is the key molecular recognition event at the heart of the adaptive and memory cellular immune responses. MHCs exhibit different selectivities for peptide, and TCRs, in their turn, exhibit different specificities for peptide-MHC complexes. Thus, the combination of selectivities displayed by MHC and TCR determines the nature and scope of peptide recognition by the immune system, and thus the detection of foreign proteins and pathogens. The T cell repertoire is large, diverse, and, in terms of its dominant recognition properties, also dependent on environmental factors, particularly infection and vaccination histories. The selectivity for peptides exhibited by an MHC molecule is, by contrast, determined solely by its molecular structure. It undergoes no somatic hypermutation, affinity maturation, or thymic selection and is, as part of a restricted set of alleles, an inherited characteristic of individual organisms. However, in humans, as in most other species, the MHC is both polygenic (there are several MHC class I and MHC class II genes) and polymorphic (there are multiple alleles of each gene) (1). Each class of MHC is represented by several loci: HLA-A, HLA-B, and HLA-C for class I and HLA-DR, HLA-DQ, and HLA-DP for class II. All MHC loci are codominant: both maternally and paternally inherited sets of alleles are expressed. The linked set of MHC alleles found on one chromosome is called a haplotype. MHCs exhibit extreme polymorphism: within the human population there are, at each genetic locus, a great number of alleles—the last IMGT/HLA database release (April 2003) lists 948 class I and 633 class II molecules (2), many of which are represented at high frequency

(>1%). MHC alleles may differ by as many as 30-aa substitutions. Such an uncommon degree of polymorphism implies a selective pressure to create and maintain it. Different polymorphic MHC alleles, of both class I and class II, have different peptide specificities: each allele binds peptides exhibiting particular sequence patterns.

The majority of polymorphic sites in class I alleles are found in the $\alpha 1$ and $\alpha 2$ domains of the mature protein. These domains form the peptide binding site (3, 4). Analysis of x-ray structures of HLA/peptide complexes identified ~35 residues within the binding site as being critical for peptide binding and therefore for T cell recognition (5–7). About 20 of them are polymorphic. Six binding pockets, denoted A through F, are formed within the binding site. Some of the pockets are nonpolar and can form hydrophobic contacts, but others contain polar atoms that can make hydrogen bonds with peptide side chains.

The peptide side chains at certain positions bind tightly to certain pockets and form primary anchors (8). The combination of two or more anchors is called a motif. It has been found experimentally that certain alleles can recognize very similar motifs (9), and thus be grouped into HLA supertypes. The classification of HLA molecules into supertypes (superfamilies) in terms of their structural features and abilities to bind peptides with common motifs (supermotifs) is an important issue, with direct implications for the development of epitope-based vaccines (10–14). Determining the peptide specificity of even a single allele is by no means a trivial undertaking, requiring prodigious experimental work (13, 14) and sophisticated theoretical analysis (15, 16). To undertake this rigorous task for over 1500 alleles is simply too intensive, in terms of labor, time, and resources, to be practical. The only viable alternative is to seek a bioinformatic approach.

At the present time, there are two groups of *in silico* classification: whole sequence-based (17, 18) and pocket-based (19–21). Both types of methods, although informed by structural knowledge, make use of protein sequences. The difference between these methods is the number of residues involved in the analyses. The pocket-based classifications have more direct, practical application to vaccine development. Motif-based categorization is a variety of

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pocket-based classification, where only pockets accepting peptide anchors are considered (9–14).

In this study, we describe a bioinformatics strategy for classifying the HLA class I molecules into supertypes, using information drawn solely from three-dimensional (3D)² protein structures. Two chemometric techniques—hierarchical clustering and principal component (PC) analysis (PCA)—were used independently on a set of 783 HLA class I molecules to identify supertypes based on structural similarities and molecular interaction fields (MIFs) calculated for the peptide binding site on HLA class I molecules. The supertypes derived by both techniques were compared and analyzed to identify a “supertype fingerprint,” i.e., a 3D structural basis for supertype classification. The identified fingerprints revealed the striking observation that only one to three amino acids are sufficient for the classification of an allele to a particular supertype.

Materials and Methods

Protein structures

The protein sequences of 783 HLA class I molecules were collected from the IMGT/HLA database (2): 229 molecules for HLA-A, 447 for HLA-B, and 107 for HLA-C. The protein molecules were modeled based on x-ray data for three reference proteins retrieved from the Research Collaboratory for Structural Bioinformatics (RCSB) protein database (22): molecule A*0201 (Protein Data Bank code: 1I4F) for the A locus (23), B*0801 (Protein Data Bank code: 1AGD) for the B locus (24), and Cw*0401 (Protein Data Bank code: 1IM9) for the C locus (25). For these proteins, all cofactors, counterions, ligands, and water molecules were removed before the modeling of the remaining 780 HLA molecules. Side chain placement of polymorphic residues was performed using SCRWL2.8 (26). As the polymorphic amino acids and the amino acids involved in the binding site are localized among the first 180 residues, the modeled molecules consisted of $\alpha 1$ and $\alpha 2$ domains only (residues 1–180). MHC nomenclature is becoming complex (2), with the recent inclusion of subsidiary numbers beyond the familiar four digit code, to include, *inter alia*, nucleic acid difference in both exon and introns which have no effect on the amino acid sequence. In such cases, only the first molecule (*xxxx01) was considered so that a set of all unique protein sequences were retained.

All chemometric modeling studies were performed on a SGI workstation (Silicon Graphics, Mountain View, CA) using the programs Sybyl (version 6.9; Tripos, St. Louis, MO), GRID (version 21; Molecular Discovery, Oxford, U.K.), and Golpe (version 4.5.12; Multivariate Infometric Analysis, Perugia, Italy). Modeled protein molecules were aligned within each locus using the initial x-ray structure as a template. Hydrogen atoms were added and Kollman charges were calculated for each molecule. For GRID/Golpe, the molecules from each locus were added as a multiple target and the GRID MIFs with different probes were calculated.

Binding site definition

Based on the x-ray structures, three different MHC binding sites were defined: one site per locus. The HLA-A binding site includes the following 35 residues: 5, 7, 9, 24, 25, 34, 45, 59, 63, 66, 67, 70, 74, 77, 80, 81, 84, 97, 99, 113, 114, 116, 123, 133, 143, 146, 147, 152, 155, 156, 159, 160, 163, 167, and 171 (5). The HLA-B binding site consisted of 37 residues: 5, 7, 8, 9, 24, 45, 59, 62, 63, 65, 66, 67, 70, 73, 74, 76, 77, 80, 81, 84, 95, 97, 99, 114, 116, 123, 143, 146, 147, 152, 155, 156, 159, 160, 163, 167, and 171 (6). The HLA-C binding site comprises 32 residues: 5, 7, 9, 22, 59, 62, 63, 66, 67, 69, 70, 73, 74, 77, 80, 81, 84, 95, 97, 99, 116, 123, 124, 143, 146, 147, 156, 159, 163, 164, 167, and 171 (7).

Hierarchical clustering

Clustering is the process of dividing a set of entities into subsets in which the members of each subset are similar to each other but different from members of other subsets (27). There have been numerous cluster methods described (28). In the present study, a hierarchical clustering using the agglomerative algorithm (27) was applied. According to this algorithm, the clusters are built from the bottom up, first by merging individual items into

clusters, and then by merging clusters into superclusters, until the final merge brings all items into a single cluster.

This method was applied as implemented in Sybyl 6.9 (Tripos). The distance between the clusters was calculated by the complete-linkage method, i.e., the distance between the most distant pair of data points in both clusters is taken into account. The last two or three levels were considered for the supertypes definition.

Comparative similarity indices analysis (CoMSIA) fields

The hierarchical clustering of the HLA class I molecules studied here is based on five properties generated by CoMSIA (29–31): steric bulk, electrostatic potential, hydrophobicity, hydrogen-bond donor, and acceptor abilities. CoMSIA is a “3D-grid” method. In the 3D-grid methods, a probe group is moved through a regular 3D grid of points in a region of interest around the target molecule. At each point, an interaction energy or similarity index between the probe and the target molecule is calculated using an empirical function (29). In CoMSIA, similarity indices are calculated using a Gaussian-type distance dependence between a probe and the atoms of the protein molecules.

In this study, CoMSIA was used as implemented in Sybyl 6.9 (Tripos). The common probe has a 1 Å radius, charge +1, hydrophobicity +1, hydrogen-bond donor and acceptor properties +1. The region was defined on the atomic positions of the amino acids belonging to the binding site. Previous studies (32–34) indicated that 3D-quantitative structure-activity relationship models based on all fields give better results than models based on each field separately or models based on different field combinations. Because of this, all five CoMSIA fields were included in the clustering process.

Principal component analysis

The PCA is a multivariate data analysis designed to represent large, multidimensional data sets in a limited, but visually interpretable, number of dimensions, usually two to five, usually referred to as PCs, such that an overview of the data is obtained. This overview may reveal groups of observations, trends, and outliers. It also uncovers the relationships between observations and variables and between the variables themselves (35). The results from PCA can be visualized on different plots—scores and loadings. Score plots (observation projections on PC) visualize groupings of the protein molecules based on their protein-probe interaction pattern. Proteins with similar interaction patterns should be clustered. Loading plots (variable projections on PC) visualize the structural features on the molecules responsible for different or similar interaction patterns.

PCA and consensus PCA (CPCA) were used as implemented in the Golpe program (Multivariate Infometric Analysis). CPCA explains the variance on a superlevel, which expresses the “consensus” of all probes in the analysis. To focus the analysis only on attractive protein-probe interactions, a maximum cutoff value of 0 kcal/mol was used. Absolute values smaller than 0.01, or with a SD below 0.01, were set to zero. Initially, PCA was applied on MIFs generated for each probe. The sum of the explained variance by the first two PCs was considered as a criterion for the probe importance. Probes with >35% explained variance remained in the final analysis. They were analyzed by CPCA. Block unscaled weights scaling was used to normalize the importance of probe interactions.

Molecular interaction fields

In this paper, the PCA was based on the MIFs computed by the GRID program (version 21; Molecular Discovery) using particular probes. The GRID MIFs method is also a 3D-grid method, but instead of similarity indices, interaction energies are calculated at each point of the grid. These energies may be displayed as 3D contours around the target molecule using computer graphics. Contours at large negative energies indicate energetically favorable binding regions for the particular probe while those at large positive energies correspond to regions from which it would be repelled.

MIFs were calculated using the GRID force field and 13 probes (Table I), 2 Å grid spacing, and dynamic side chain treatment (GRID directive MOVE = 1). The GRID box was defined to include only the amino acids of the binding site. The GRID probes were chosen to represent all relevant interactions (hydrophobic, charge-charge, hydrogen-bond donor/acceptor) and to cover the chemical groups presented by naturally occurring amino acids.

Results

HLA-A supertypes

Hierarchical clustering. The hierarchical clustering on CoMSIA fields for 229 HLA-A molecules is given in Fig. 1, *upper left panel*. Three well-defined clusters were distinguished. The first

² Abbreviations used in this paper: 3D, three dimensional; PC, principal component; PCA, PC analysis; MIF, molecular interaction field; CoMSIA, comparative similarity indices analysis; CPCA, consensus PCA.

Table I. GRID probes used in this study^a

Name	Chemical group	HLA-A	HLA-B	HLA-C
OH2	Water	X	X	X
DRY	The hydrophobic probe	X	X	X
H	Hydrogen	X	X	
C3	Methyl CH3 group	X		
C1=	sp2 CH aromatic or vinyl	X		
N:#	sp N with lone pair	X	X	X
N:=	sp2 N with lone pair	X	X	
N1	Neutral flat NH, e.g. amide		X	
N2 ⁺	sp3 amine NH2 cation	X	X	
O1	Alkyl hydroxy OH group			X
OH	Phenol or carboxy OH	X	X	X
O	sp2 carbonyl oxygen	X	X	X
S1	Neutral SH group			X

^a In the last three columns are given the probes used in the final CPCA for each locus.

cluster, on the left side of the dendrogram, consisted of *02, *25, *26, *3401, *3405, *4301, *66, *6802, *6815, *6823, and *6901 alleles. For more clarification, the alleles from each supertype are listed in Table II. Data from the immunological literature classifies alleles *02, *6802, and *6901 molecules as the *A2 supertype* (9–12). For consistency with the literature, we adopted this name for the cluster. The central cluster was classified as *A24 supertype*, because it includes alleles *23 and *24 (Table II). This is a small, but quite well distinguished, cluster. It is closer to the A3 supertype than to A2. The last cluster is the biggest HLA-A supertype, consisting of a great variety of alleles. As alleles *03, *11, *31, and *33 were classified here (Table II), we named it the *A3 supertype* in accordance with literature precedent (9–12).

Principal component analysis. Golpe PCA was applied to multivariate GRID descriptors to uncover differences in the binding sites with respect to their probe interaction pattern. After superimposing the 3D structures of 229 HLA-A molecules using as a template the A*0201 allele (Protein Data Bank code: 1I4F), their binding sites were characterized by the interaction energies to 13 functional groups (probes). The variance explained by the first two PCs for every probe was calculated by Golpe PCA and those with >35% of the variance explained by the first two PCs were selected for CPCA (Table I). The CPCA gave a satisfactory two-component model. The first PC explains 25% of the variance, while the second adds 17%.

The CPCA scores plot is given in Fig. 1, *upper right panel*. Each point represents a single HLA-A molecule. Three clusters were defined by the first two components. On the *upper middle/right part* of the plot are molecules defined as the A3 supertype by the hierarchical clustering (Table II). Compared with the hierarchical clustering, 28 more molecules were classified here: A*25, A*26, A*4301, and A*66. According to the hierarchical classification they belong to the A2 supertype. On the *middle lower part* are clustered molecules from the A2 supertype. Molecules from the A24 supertype are situated in the *upper left quadrant*.

The CPCA loadings plots contain information about the structural features responsible for the discrimination between HLA-A supertypes. They are represented as isocontour plots for each PC. Positive contours indicate regions with positive contributions to the PC, negative contours correspond to negative PC values. The interaction fields are localized in the vicinity of four positions: 9, 97, 114, and 116. The C3/PC1 plot is shown in Fig. 1, *lower left panel*. The other plots are not shown because they are similar. The C3 probe accounts for steric and hydrophobic interactions. The black area near position 9 indicates bulky and/or hydrophobic regions favored by the

A3 supertype. The gray areas near positions 97, 114, and 116 indicate regions where bulky/hydrophobic amino acids are disfavored by A24. Indeed, A3 molecules have Phe and Tyr at position 9, whereas all A24 proteins have Ser. Furthermore, A24 supertype has the unique combination of Met⁹⁷, His¹¹⁴, and Tyr¹¹⁶.

HLA-B supertypes

Hierarchical clustering. Three clusters are distinguished for the HLA-B locus after hierarchical clustering on 447 HLA-B molecules (Fig. 2, *upper left panel*). These three clusters define three B supertypes. The first cluster from the left was defined as the *B44 supertype* as B*44 is the serotype with the largest of alleles here (Table III). The B44 supertype also includes molecules B*0802, B*13, several B*15, B*1809, B*2701, B*2702, B*38, B*4013, B*4019, B*44, B*4704, B*49, B*51, B*52, B*53, B*57, B*58, and B*5901. The middle cluster was christened the *B27 supertype* because most B*27 alleles were included there. Other molecules belonging to this supertype are B*0713, B*1309, B*15, B*1812, B*27, B*3513, B*3516, B*3528, B*37, B*3803, several B*39, B*40, B*41, B*4409, B*4431, B*45, B*46, B*47, B*48, B*50, B*5608, B*6702, and B*7805 (Table III). The last HLA-B cluster includes the following alleles: B*07, B*08, B*14, several B*15, B*18, B*2723, B*35, B*39, B*4008, B*4025, B*42, B*4806, B*5303, B*5305, B*54, B*55, B*56, B*6701, B*7301, B*78, B*81, B*82, and B*83 (Table III). The largest serotype here is B*07 and this cluster was named the *B7 supertype*.

Principal component analysis. All HLA-B molecules were aligned using as a template B*0801 (Protein Data Bank code: 1AGD). Six probes were found to explain >35% of the variance in the first two PCs (Table II). Initially, no consensus in the probe-protein interaction patterns was found by CPCA. To reduce the number of field variables, a region selection tool in Golpe was applied. The amino acids of the binding site were used as a template, and all data points within 4 Å were retained. This gave three well-defined clusters on the CPCA scores plot with 37% of the variance explained by the first two PCs (Fig. 2, *upper right quadrant*). The cluster on the *upper right quadrant* consisted mainly of B*27 molecules (Table III). The cluster on the *lower right quadrant* includes alleles from the B44 supertype and the big cluster on the *left side* comprises molecules belonging to the hierarchical B7 supertype. The comparison between the classifications made by hierarchical clustering and CPCA showed that 305 of 447 molecules (68%) are classified in the same supertype. The results for each supertype are: 173 common for B7, 24 for B27, and 108 for B44.

The CPCA loading plots identify two important areas of interaction (Fig. 2, *lower left panel*). The former area is localized near positions 63 and 66. Both residues participate in pockets A and B within the peptide binding site. Position 63 is polymorphic, 66 is conserved. There are two optional amino acids for position 63: Glu and Asn (only B*0810 has with Asp⁶³). The latter area of interaction is near positions 77 and 81, both parts of pocket F. Asn, Ser, and Asp are found at 77, Leu and Ala at 81.

HLA-C supertypes

Hierarchical clustering. Two clusters were generated after hierarchical clustering on CoMSIA fields for 107 HLA-C molecules (Fig. 3, *upper left panel*, Table IV). The cluster on the *left side* of the dendrogram consists of Cw*02, Cw*0307, 08, 10, 15, Cw*04, Cw*05, Cw*06, Cw*0701, 06, 07, 09, 16, 18, Cw*1204, 05, 08, Cw*15, Cw*1602, Cw*1701, 02, 03, Cw*1801 and 02. We named this cluster supertype C4. The cluster on the *right side* includes Cw*01, Cw*0302, 03, 04, 05, 06, 09, 11, 12, 13, 14, 16, Cw*0702, 03, 04, 05, 08, 10, 11, 12, 13, 14, 15, 17, Cw*08, Cw*1202, 03, 06, 07, Cw*14, Cw*1601 and 04. We called this cluster *supertype C1*.

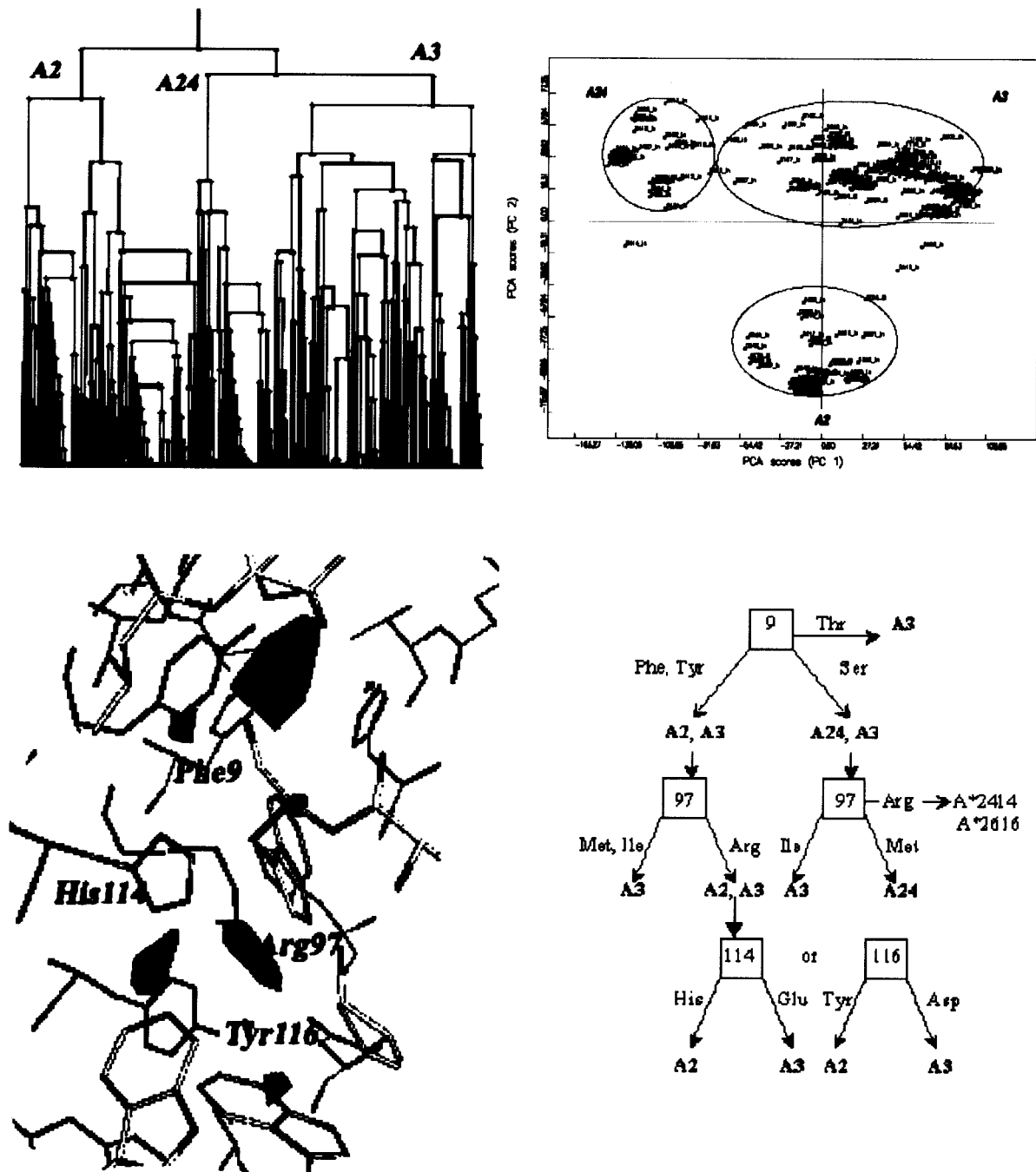


FIGURE 1. HLA-A supertypes. *Upper left panel*, Hierarchical clustering. *Upper right panel*, CPCA scores plot. *Lower left panel*, CPCA loadings plot of PC1 for C3 probe. X-ray structure of HLA-A*0201 is used to show the binding site amino acids close to the fields. The black and gray areas describe positions where differences between alleles exist. *Lower right panel*, Guide for HLA-A supertype determination.

Principal component analysis. Allele Cw*0401 (Protein Data Bank code: 1IM9) was used as a template in the alignment of HLA-C molecules. Eight probes were selected for the CPCA on HLA-C molecules (Table II). Two clusters could be defined in the two-dimensional CPCA scores plot (Fig. 3, *upper right panel*). The first PC explains 21% of the variance, the second 15%. The clusters are quite diffuse with many outliers. PC2 is more important for the clustering and that is why the loading plots were created on PC2. The clusters are in a good agreement with the hierarchical classification (Table IV). Only eight alleles were misclassified: Cw*0308, Cw*0310, Cw*0701, Cw*0706, Cw*0716, Cw*0718, Cw*1208, and Cw*1404.

All loading plots highlight two areas (one positive and one negative) inside pocket F: positions 70, 74, 77, and 81 (Fig. 3, *lower left panel*), of which only position 77 is polymorphic. Ser and Asn are the optional amino acids at this position. The cluster which makes positive contributions to PC2 (C1 supertype) favors interactions with a water molecule, i.e., Ser⁷⁷ is preferred. The cluster making negative contributions (C4 supertype) favors a less polar residue here, like Asn⁷⁷.

Discussion

Two independent chemometric techniques were applied to the 3D structures of 783 HLA molecules to classify them into supertypes.

Table II. *HLA-A supertypes*

Supertype	Motif-based ^a	Hierarchical Clustering	CPCA	Fingerprint
A1	0101 2501 2601, 02 3201			
A2	0201-07 6802 6901	0201-60 2501-04 2601-18 3401, 05 4301 6601-04 6802, 15, 23 6901	0201-60 without 04, 17, 57 6802, 15, 23 6901	Tyr ⁹ /Phe ⁹ Arg ⁹⁷ His ¹¹⁴ and Tyr ¹¹⁶
A24	2301 2402-04 3001-03	2301-09 2402-38	2301-09 2402-38	Ser ⁹ Met ⁹⁷
A3	0301 1101 3101 3301 6801	0101-09 0301-10 1101-14 2901-07 3001-12 3101-09 3201-07 3301-06 3402-04 3601-04 6801-22 without 02, 15 7401-09 8001	0101-09 0301-10 1101-14 2501-04 2601-18 2901-07 3001-12 3101-09 3201-07 3301-06 3401-05 3601-04 4301 6601-04 6801-23 without 02, 15 7401-09 8001	Tyr ⁹ /Phe ⁹ /Ser ⁹ Ile ⁹⁷ /Met ⁹⁷ Glu ¹¹⁴ and Asp ¹¹⁶

^a Refs. 9–12.

The former technique—hierarchical clustering using CoMSIA fields—classifies the molecules on the basis of structural similarities in terms of steric bulk, electrostatic potential, hydrophobicity, and hydrogen-bond donor and acceptor abilities. The latter technique uses CPCA on probe-ligand interaction fields and classifies the proteins according to their interaction patterns. Seventy-seven percent of all molecules were classified in the same supertype by both techniques. Where data is available, our classification seems in good agreement with motif-based supertype classification (10–12, 36).

The CPCA loading plots indicate the differences between the supertypes. Taking into account these differences, guides were created for supertype determination within each locus. Thus, every known or new HLA class I molecule could be classified on the basis of only one to three residues. The combination of these residues for each supertype we called a supertype fingerprint. As five different properties (steric bulk, electrostatic potential, hydrophobicity, hydrogen-bond donor, and acceptor affinities) are considered at the same time in the hierarchical clustering, the guides were designed to follow the hierarchical classification.

Four HLA-A supertypes are defined by Sette et al. (12), based on common binding motifs: A1, A2, A3, and A24. The A1 supertype consists of A*0101, A*2501, A*2601, A*2602, and A*3201 (12). The A2 supertype includes A*0201-07, A*6802, and A*6901 (9). The A3 supertype comprises A*0301, A*1101, A*3101, A*3301, and A*6801 (36). The A24 supertype was defined to include A*2301, A*2402-04, and A*3001-03 (12). Our previous quantitative structure-activity relationship (QSAR) studies confirmed and extended the supermotifs for A2 and A3 supertypes (37, 38). The A*6802 molecule was found to be an intermediate

allele lying between the A2 and A3 supertypes: in anchor position 2 it is closer to A3 and in anchor position 9 it is closer to A2 (38).

The hierarchical clustering and PCA classification made in the present study on 229 HLA-A molecules gave an 88% consensus, i.e., 201 HLA-A molecules are classified in the same manner by both methods. Good agreement exists with the motif-based supertypes, except for of A*0101, A*2501, A*2601, 02, A*3001–03, and A*3201 molecules. They were classified as A1 or A24 supertypes by Sette et al. (12). Our classification puts them into the A2 or A3 supertypes (Table II).

The CPCA loadings plots (Fig. 1, lower left panel) draw attention to four positions in the HLA-A alleles: 9, 97, 114, and 116. The amino acid at position 9 takes part in the formation of pocket B, one of the most specific pockets of the binding site. All A2 supertype, and most of the A3 supertype, alleles have Phe or Tyr at this position, making pocket B shallow, while A24 supertype alleles have Ser there. Position 9 of the MHC molecules corresponds to the primary anchor position 2 of the binding peptide. The binding motifs for A2 and A3 supertypes have common anchors at position 2: Leu, Ile, Met, Val, and Ala, in contrast with A24 motif where Tyr is preferred. Residue 97 forms part of pockets C and E, corresponding to peptide positions 6 and 7, respectively. The amino acid at position 114 forms pockets D and E. Finally, residue 116 is part of pocket F, where the peptide C-terminal binds. The great difference between A2 and A3 binding motifs concerns anchors at position 9 (C-terminal): A2 alleles prefer Val, while A3 prefers Arg. Bulky residues like Tyr¹¹⁶ in A2 alleles restrict the size of pocket F and thus peptides bearing small hydrophobic amino acids are preferred there. In contrast, small negatively

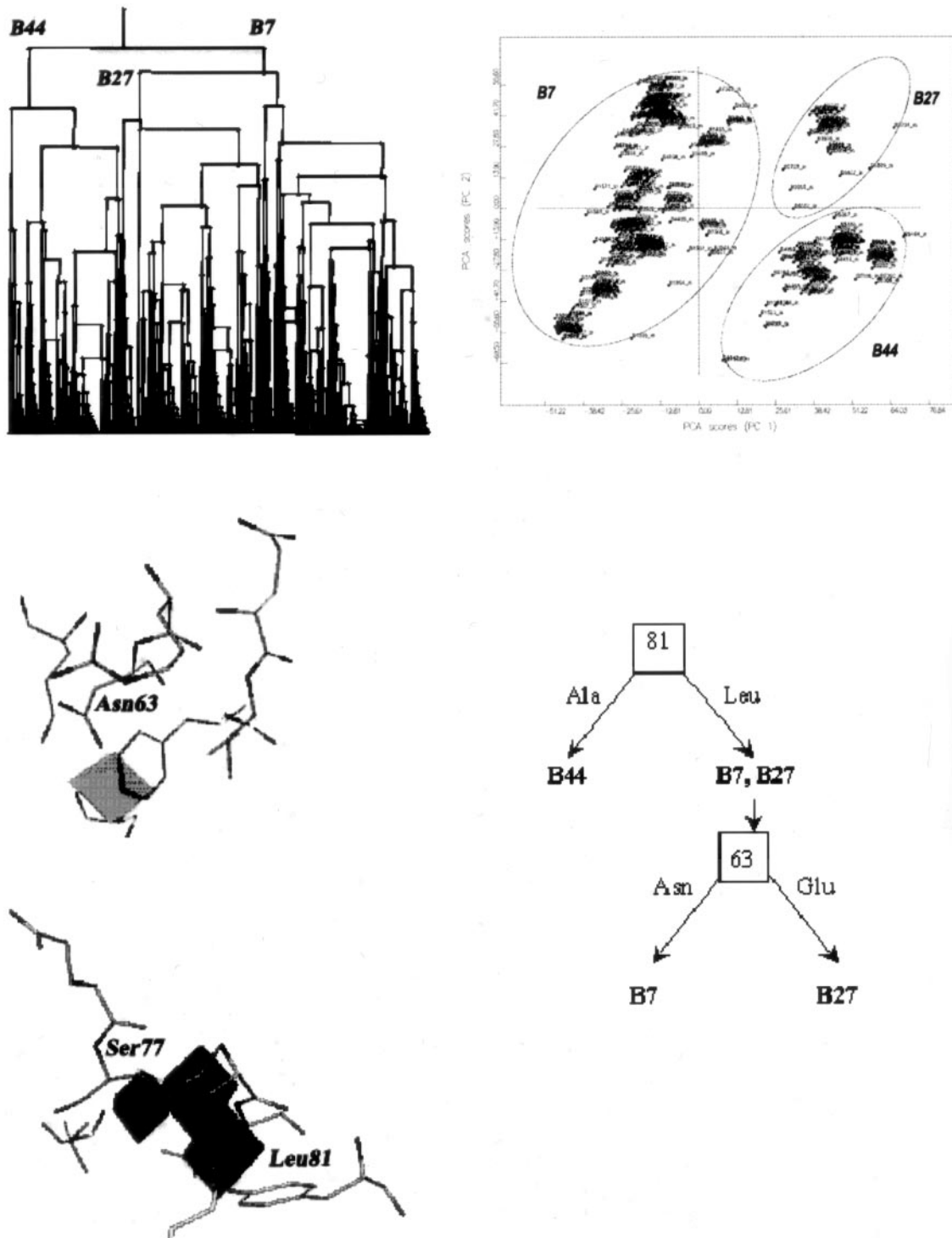


FIGURE 2. HLA-B supertypes. *Upper left panel*, Hierarchical clustering. *Upper right panel*, CPCA scores plot. *Lower left panel*, CPCA loadings plots of PC1 for DRY probe. X-ray structure of HLA-B*0801 is used to show the binding site amino acids close to the fields. The black and gray areas describe positions where differences between alleles exist. *Lower right panel*, Guide for HLA-B supertype determination.

charged residues like Asp¹¹⁶ in A3 alleles are more compatible with larger, positively charged amino acids such as Arg.

Considering positions 9, 97, 114, and 116, we identified a guide for HLA-A supertype fingerprint determination (Fig. 1, *lower right panel*). Starting at residue 9, Phe, Tyr or Thr here indicates A2 or A3 supertypes, Ser leads to A24 and certain A3 alleles. Next is position 97, which discriminates between A3 (Met⁹⁷ or Ile⁹⁷) and A2 (Arg⁹⁷), and between A3 (Ile⁹⁷) and A24 (Met⁹⁷). When position 97 is Arg, an additional step is required: residue 114 or 116.

His¹¹⁴ and Tyr¹¹⁶ are characteristic of A2 molecules, while Glu¹¹⁴ or Asp¹¹⁶ selects for A3. Hence, the fingerprint for the A2 supertype is Tyr⁹/Phe⁹, Arg⁹⁷ and His¹¹⁴ or Tyr¹¹⁶; for A3–Tyr⁹/Phe⁹/Ser⁹, Ile⁹⁷/Met⁹⁷ and Glu¹¹⁴ or Asp¹¹⁶; for A24–Ser⁹ and Met⁹⁷.

For HLA-B, hierarchical clustering and CPCA define three supertypes: B7, B27, and B44 with a consensus of 68%. The most questionable is the B27 supertype (only 24 common alleles). Most of the alleles defined as B27 by hierarchical clustering are classified as B7 or B44 by the CPCA. Sette et al. (12) define five motif-based

Table III. *HLA-B supertypes*

Supertype	Motif-Based ^a	Hierarchical Clustering	CPCA	Fingerprint		
B44	37	0802	0802	Ala ⁸¹		
	40012	1301-1311 without 09	1301-1311 without 09			
	4006	1513, 16, 17, 23, 24, 36, 67	1513, 16, 17, 23, 24, 36, 67			
	41	1809	1809			
	44	2701, 02	3805			
	45	3801-3809 without 03	4402-33 without 09			
	47	4013, 4019	4701-04 without 02			
	49	4402-4433 without 09, 31	4901-03			
	50	4704	5101-34			
		4901-03	5201-05			
		5101-34	5301-09			
		5201-05	5607			
		5301-09 without 03, 05	5701-09			
		5701-09	58-07			
		5801-07	5901			
		5901				
		0713	0727			
	B27	1401-02	1309		2701-25 without 08, 12, 18	Glu ⁶³ Leu ⁸¹
		1503, 09, 10, 18	1501-1575 without these in B7 and B44		3701-04	
2701-08		1812	3801-09			
3801, 02		2703-2725	4013, 19, 28			
3901-04		3513, 16, 28				
4801, 02		3701-05				
7301		3803				
		3902, 08, 13, 22, 23				
		4001-44 without 08, 13, 19, 25				
		4101-06				
		4409, 31				
		4501-06				
		4601, 02				
		4701-03				
		4801-07				
		5001-04				
		5608				
		6702				
		7805				
B7	07	0702-31 without 13	0702-31 without 0727	Asn ⁶³ Leu ⁸¹		
	35	0801-17 without 02	0801-17 without 02			
	51	1401-06	1309			
	53	1502, 08, 09, 10, 11, 15, 18, 21, 29,	1401-06			
	54	37, 44, 51, 52, 55, 64, 72	1501-75 without 13, 16, 17,			
	55	1801-18 without 09, 12	23, 24, 36, 67			
	56	2723	1801-18 without 09			
	67	3501-45 without 13, 16, 28	2708, 12, 18			
	78	3901-27 without 02, 08, 13, 22,	3501-45			
		23	3705			
		4008, 25	3904			
		4201-04	4101-06			
		4806	4201-04			
		5303, 05	4409			
		5401, 02	4501-06			
		5501-12	4601, 02			
		5601-11 without 08	4702			
		6701	4801-07			
		7301	5001-04			
		7801-04	5401, 02			
		8101	5501-10			
		8201, 02	5601-11 without 5607			
		8301	6701, 02			
		7301				
		7801-05				
		8101				
		8201, 02				
		8301				
B58	1516, 17					
	5701, 02					
	58					
B62	1301-02					
	1501, 02, 06, 12, 13, 14,					
	19, 21					
	4601					
	52					

^a Refs. 10–12, 36.

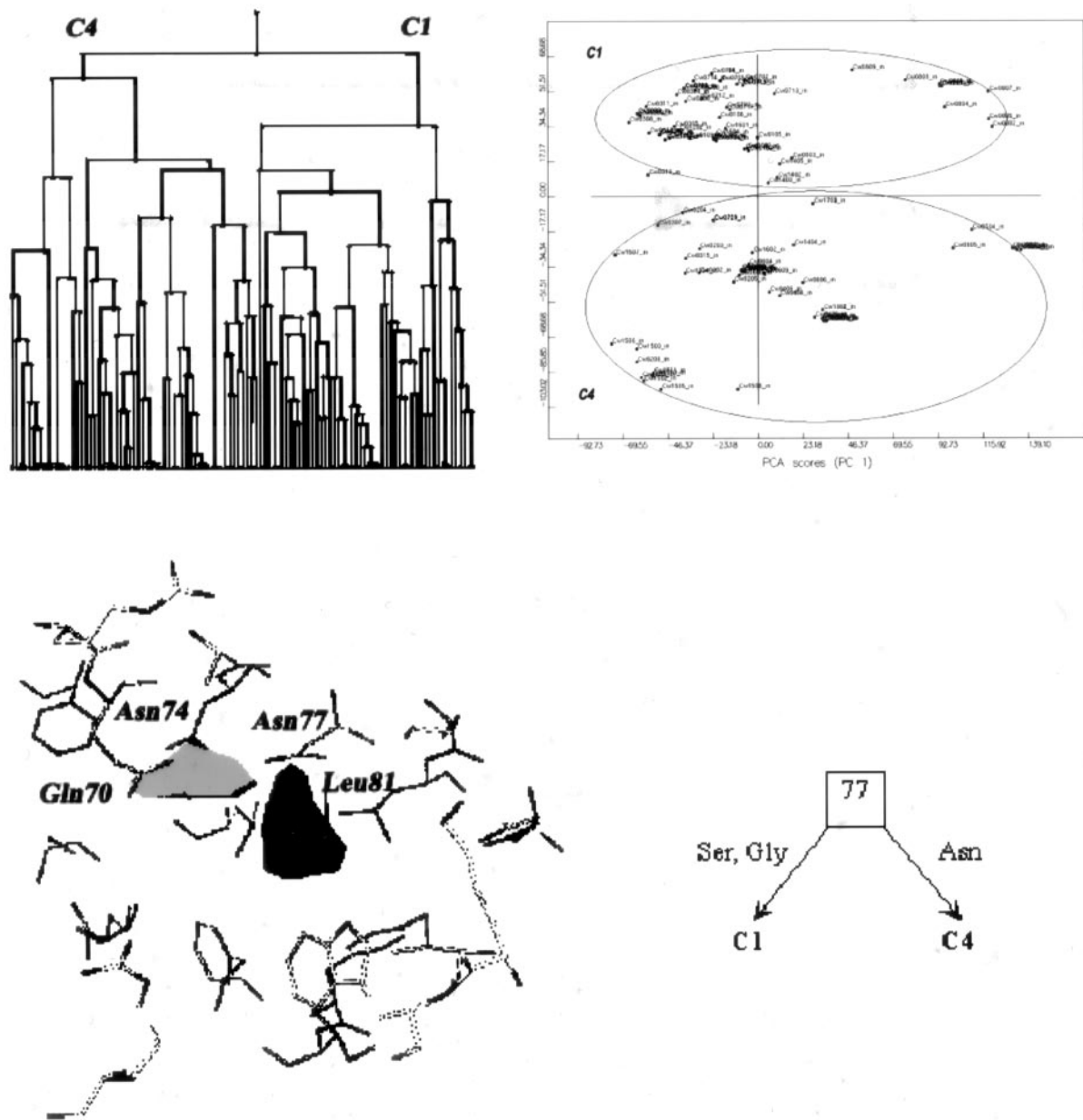


FIGURE 3. HLA-C supertypes. *Upper left panel*, Hierarchical clustering. *Upper right panel*, CPCA scores plot. *Lower left panel*, CPCA loadings plot of PC2 for H₂O probe. X-ray structure of HLA-Cw*0401 is used to show the binding site amino acids close to the fields. The black and gray areas describe positions where differences between alleles exist. *Lower right panel*, Guide for HLA-C supertype determination.

HLA-B supertypes: B7, B27, B44, B58, and B62 (Table III). Sette's B58 supertype was clustered as a part of B44 in the present study, and B62 as part of B7, B27, or B44.

The positions highlighted in the CPCA loading plots allowed us to draw a guide for HLA-B supertype fingerprint determination (Fig. 2, *lower right*), analogous to the HLA-A guide. Starting at position 81, the HLA-B molecules could be divided into two groups: the former group has Ala, the latter has Leu. All B44 alleles have Ala⁸¹, while B7 and B27 have Leu⁸¹. The next is position 63. B27 alleles have Glu⁶³ and B7 has Asn⁶³. Thus, the fingerprint for B7 affiliation is Asn⁶³ and Leu⁸¹; for B27–Glu⁶³ and Leu⁸¹; for B44–Ala⁸¹.

At the present time, information about HLA-C supertypes is not available in the literature. The present study identifies two supertypes, which we call C1 and C4, respectively (Table IV, Fig. 3, *lower left and right panels*). Only position 77 was found to be

important for this classification. Molecules with Ser⁷⁷ belong to C1 supertype, these with Asn⁷⁷ to C4. Position 77 is a key position in protein molecules from the C locus. It takes part in the formation of pockets C and F, and the killer Ig-like receptor binding site (7). There is 93% consensus between the hierarchical and CPCA-based classifications for HLA-C molecules (Table IV).

The small number of supertype-determining MHC sequence positions delineated by our analysis all equate to solvent-exposed residues contributing to the peptide binding site. If, for simplicity, we assume that, over time, random mutations will occur at the nucleic acid level—so called single nucleotide polymorphisms—then an MHC will, in the absence of other selective pressures, accumulate point mutations throughout its sequence. Some will be “silent” mutations, which do not change amino acid identity, and others, often called mis-sense mutations, which will alter the phenotypic amino acid. For solvent inaccessible residues buried in the

Table IV. *HLA-C supertypes*

Supertype	Motif-Based	Hierarchical Clustering	CPCA	Fingerprint
C1	No data	0102-09	0102-09	Ser ⁷⁷ /Gly ⁷⁷
		0302-16 without 07, 08, 10, 15	0302-16 without 7, 15	
		0702-18 without 01, 06, 07, 09, 16, 18	0701-18 without 07, 09	
		0801-09	0801-09	
		1202-07 without 04, 05, 08	1202-08 without 04, 05	
		1402-05	1402-05	
		1601, 04	1601, 04	
		0202-06	0202-06	
		0307, 08, 10, 15	0307, 15	
		0401-10	0401-10	
C2	No data	0501-06	0501-06	Asn ⁷⁷
		0602-09	0602-09	
		0701, 06, 07, 09, 16, 18	0707, 09	
		1204, 05, 08	1204, 05	
		1502-11	1404	
		1602	1502-11	
		1701-03	1602	
		1801, 02	1701-03	
			1801, 02	

protein core, such changes will only rarely be advantageous and, on the basis of stability, be selected against. Some mutations will have little or no functional consequence, but those interfering with, say, binding to β_2 -microglobulin, or to TCRs, or to bound peptides will affect function. In terms of peptide binding, certain amino acid mutations will have a significant effect on the peptide binding site microenvironment, altering residue size, hydrophobicity, or charge. Within the context of the cellular Ag presentation pathway, class I MHCs will select for changes that moderate the size, shape, and physical-chemical environment within the characteristic binding sites exhibited by different supertypes. MHC supertypes are thought to arise through both divergent and convergent molecular evolution. Such processes will concentrate acceptable mutations at certain sequence positions where conflicting constraints can best be balanced. Our analysis, though it contains no overt phylogenetic component, and indeed uses no direct amino acid sequence information in calculating similarities between binding sites, nonetheless allows us to identify crucial sequence positions for the determination of peptide specificity.

Two principal advantages of the present classification should be highlighted. First, this approach is based on the whole binding site, not on particular pockets. There is a lot of experimental data to show that peptide anchors are necessary, but not sufficient, for good binding. There are several secondary anchors (positions 1, 3, 6, and 7) which are also responsible for high affinity (39). Considering the whole binding site provides us with a wider, and clearer, overview of the interactions within it. Second, the approach classifies all currently known HLA molecules, an experimental task that is presently impossible. The resulting supertype fingerprints are both simple and easy to apply for every known, or newly discovered, HLA class I molecule, and, potentially, for MHCs from other species. The classification of alleles into supertypes is useful for defining promiscuous peptides able to bind to numerous alleles (40, 41) and for identifying proper population coverage of peptide-based vaccines (42–44). The greatly increased scope of our analysis opens the way to a broad definition of MHC specificities, ensuring cross-reactive peptide vaccines able to cover the whole human population can be designed.

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