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REVIEW

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Nine major HLA class I supertypes account for the vast preponderance of HLA-A and -B polymorphism

Abstract Herein, we review the epitope approach to vaccine development, and discuss how knowledge of HLA supertypes might be used as a tool in the development of such vaccines. After reviewing the main structural features of the A2-, A3-, B7-, and B44- supertype alleles, and biological data demonstrating their immunological relevance, we analyze the frequency at which these supertype alleles are expressed in various ethnicities and discuss the relevance of those observations to vaccine development. Next, the existence of five new supertypes (A1, A24, B27, B58, and B62) is reported. As a result, it is possible to account for the predominance of all known HLA class I with only nine main functional binding specificities. The practical implications of this finding, as well as its relevance to understanding the functional implication of MHC polymorphism in humans, are discussed.

Key words Vaccines · Epitopes · HLA · Supertypes · Polymorphism · MHC

The epitope approach to vaccine development

Over the past decade, a detailed understanding of how T cells recognize antigen has emerged. The complex of a major histocompatibilty complex (MHC) molecule and a peptidic antigen acts as the ligand recognized by MHC-restricted T cells (Garcia et al. 1999; Germain and Margulies 1993; Yewdell and Bennink 1999). The study of single amino acid substituted antigen analogs and the sequencing of endogenously bound, naturally processed peptides has identified critical residues required for allele-specific binding to MHC molecules (see, e.g., Engelhard 1994; Garcia et al. 1999; Sinigaglia and Hammer 1994). X-ray crystallographic analysis of MHC-peptide complexes revealed allele specific pockets within the peptide binding cleft of MHC molecules which accommodate specific peptide side chains (Fremont et al. 1992; Guo et al. 1992; Madden et al. 1992; Matsumura et al. 1992; Saper et al. 1991).

Accordingly, the definition of class I and class II allele-specific MHC binding motifs allowed identification of regions within a protein that have the potential of binding particular MHC alleles (Engelhard 1994; Kast et al. 1994; Sette and Grey 1992; Sinigaglia and Hammer 1994). A variety of assays to detect and quantitate the affinity of peptide-MHC binding have also been established (Engelhard 1994; Joyce and Natheson 1994; Sette and Grey 1992; Sidney et al. 1998), and the threshold of affinity associated with generation of immune responses has also been elucidated (Schaeffer et al. 1989; Sette et al. 1994; Southwood et al. 1998). Thus, by a combination of motif searches and MHC-peptide binding assays, potential candidates for epitope-based vaccines can now be identified rapidly and accurately.

The epitope approach to vaccine development offers several potential advantages. These include not only a more potent response than that obtained by the use of whole antigens (Ishioka et al. 1999), but also control over qualitative aspects of the immune response. By the selection of appropriate epitopes, broad responses simultaneously targeting multiple dominant and subdominant epitopes can be induced (Oukka et al. 1996; Tourdot et al. 1997). The breadth of an immune response is thought to represent a crucial factor in control and /or resolution of HIV and HCV infection (Cooper et al. 1999; Couillin et al. 1994; McMichael and Phillips 1997; Missale et al. 1996). Accessing subdominant specificities might be of particular value in the case of tumor antigens, where self-tolerance might have inactivated T cells recognizing the most dominant specificities (Disis et al. 1996; Feltkamp et al. 1995). The epitope approach also allows focusing of immune responses against multiple conserved epitopes, a factor of

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crucial importance in the case of rapidly mutating pathogens, such as HIV and HCV (Borrow et al. 1997; Cooper et al. 1999; Goulder et al. 1997a, b; Nowak et al. 1995; Price et al. 1997; Weiner et al. 1995).

By combining epitopes derived from multiple antigens into a single immunogen, (Ishioka et al. 1999), it might be possible to overcome the limited capacity of certain delivery systems. By the same strategy, it is also possible to guard against low efficacy due to a lack or loss of antigen expression (Kawashima et al. 1998), a factor of importance when a single protein antigen is targeted for vaccine development. Furthermore, epitope based vaccines can also be analogued to increase potency and break tolerance, as highlighted by a number of different studies (Ahlers et al. 1997; Parkhurst et al. 1996; Sarobe et al. 1998; Tsai et al. 1997).

It is important to emphasize that the use of epitopes isolated from the context of the antigen of origin can overcome potential safety concerns, as exemplified by the case of the HPV E6 and E7 antigens whose expression is clearly associated with cervical carcinoma (Crook et al. 1989; Hawley-Nelson et al. 1989).

In parallel, recent evidence has been provided to validate the epitope approach for treatment and/or prevention of numerous different types of disease. For example, vaccination with either dominant or subdominant epitopes has been shown to protect against acute or chronic viral infection in systems such as influenza or LCMV (An et al. 1997; Oukka et al. 1996; van der Most et al. 1996). A variety of studies have also validated epitope-based vaccines as a strategy to address parasitic and microbial infections (Le et al. 1998; Wang et al. 1996), and cancer (Iwasaki et al. 1998; Mayordomo et al. 1996; Melief et al. 1996; Morgan et al. 1998; Vierboom et al. 1997). For example a tumor epitope previously engineered for high HLA binding (Parkhurst et al. 1996) was effective for treatment of human melanoma when delivered in a mineral oil emulsion in the presence of IL2 (Rosenberg et al. 1998).

In conclusion, our understanding of the role played by specific residues in the presentation peptide epitopes by class I and class II HLA molecules has greatly advanced in the past several years. A body of knowledge enhancing the ability to identify and design peptides that bind across many HLA types has emerged, and could greatly facilitate the development of vaccines for the prevention and treatment of important infectious diseases or neoplasias.

The discovery of HLA-A2-, -A3-, and -B7-supertypes: a quick review

The large degree of HLA polymorphism is a factor to be taken into account in the development of epitope-based vaccines. To address this factor, epitope selection encompassing identification of peptides capable of binding multiple HLA molecules can be utilized (Sette and Sidney 1998; Sidney et al. 1996b). Our studies have identified several HLA supermotifs, each of which corresponds to the ability of peptide ligands to bind several different HLA alleles. The HLA alleleic variants that bind peptides possessing a particular HLA supermotif are collectively referred to as an HLA supertype (Sette and Sidney 1998; Sidney et al. 1996a, b, c).

Early studies defined the motifs recognized by some of the most common HLA types (DiBrino et al. 1993a, b; Falk et al. 1994; Kubo et al. 1994; Zhang et al. 1993). It was found that HLA-*A*0301* and -*A*1101* recognized very similar motifs, leading to the hypothesis that a significant overlap might exist amongst their peptide binding repertoires. This hypothesis was verified by a study which also demonstrated that the *A*0301* and *A*1101* repertoires overlapped with those of *A*3101*, *A*3301*, and *A*6801* (Sidney et al. 1996a). This group of alleles, characterized by similar peptide specificity, was defined as the A3-supertype. Similarly, a significant overlap in peptide binding repertoires was demonstrated amongst several serologically distinct HLA-B alleles (*B*0702*, *B*3501*, *B*5101*, *B*5301*, and *B*5401* (Sidney et al. 1995, 1996c), and among different A2-subtypes (del Guercio et al. 1995; Fruci et al. 1993; Sidney et al. 1997). It has also been noted that various HLA-B alleles share a main anchor specificity that overlaps with that of HLA-*B*44* (Sidney et al. 1996b). This work resulted in the definition of the B7-, A2- and B44-supertypes, which has been reviewed in detail elsewhere (Sette and Sidney 1998; Sidney et al. 1996b). Following is a quick summary of the crucial features of the A3-, A2-, B44-, and B7-supertypes.

The A2-supertype includes, *A*0201*, *A*0202*, *A*0203*, *A*0204*, *A*0205*, *A*0206*, *A*0207*, *A*6802*, and *A*6901*. These alleles recognize peptides of about 9 or 10 residues in length which bear small or aliphatic hydrophobic residues (L, I, V, M, A, T, or Q) at position 2 and L, I, V, M, A, or T at the C- terminal position. The B pocket of A2-supertype molecules is characterized by a consensus motif including residues (where the subscript indicates MHC residue position) F/Y_9, M_{45}, E/N_{63}, K/N_{66}, V_{67}, H/Q_{70}, and Y/C_{99}. Similarly, the A2-supertype F pocket is characterized by a consensus motif including residues D_{77}, T_{80}, L_{81}, and Y_{116} (del Guercio et al. 1995; Sidney et al. 1996b).

The A3-supertype includes the *A*0301*, *A*1101*, *A*3101*, *A*3301*, and *A*6801* alleles (Sidney et al. 1996a, b). A3-supertype molecules recognize a broad motif characterized by A, V, I, L, M, S, or T in position 2, and R or K at the C-terminus (DiBrino et al. 1993a; Falk et al. 1994; Kubo et al. 1994; Maier et al. 1994; Sidney et al. 1996a). Peptide lengths of 9 to 10 amino acids have been most frequently reported, although longer peptides can bind, and sometimes be recognized, in the context of A3-supertype molecules. The B pocket of A3-supertype molecules is characterized by the consensus motif of M_{45}, N/K_{66}, M/V_{67}, Q/H_{66}, and Y_{99}. This structural motif is similar to that of A2-supertype B pockets and is in good agreement with the largely overlapping B-pocket specificity of A2- and A3-super-

type alleles. The F pocket of A3-supertype molecules is characterized by D_{77}, T_{80}, L_{81}, and D_{116}. The dominant presence of a negatively charged residue (D) in positions 77 and 116 correlates with the specificity of A3-supertype alleles for peptides with a positively charged C-terminus.

The B7-supertype, as originally described by Sidney and co-workers (1995, 1996b, c), included *B*0702*, *B*3501-03*, *B*51*, *B*5301*, and *B*5401*. Additional data for *B*0703-05*, *B*1508*, *B*5501-02*, *B*5601-02*, *B*6701*, and *B*7801* indicated that these alleles should also be included within the B7-supertype (Barber et al. 1995, 1997; Rammensee 1995). B7-supertype molecules share a peptide binding specificity for P in position 2 and a hydrophobic aliphatic (A, L, I, M, or V) or aromatic (F, W, or Y) residue at the C-terminal position of their peptide ligands. Modeling and X-ray crystallographic studies of the structure of B7 and *B*3501* have been published (Huczko 1993; Smith et al. 1996a, b), offering insights into the specificity of B7-supertype molecules. Structurally, the B7-supertype molecules share a B pocket consensus motif of Y_9, N_{63}, I_{66}, F/Y_{67}, N/Q_{70}, and Y_{99}. By contrast, no discrete B7-supertype F pocket consensus motif has yet been defined.

Finally, the B44-supertype was defined on the basis of a shared specificity for peptides with negatively charged (D, E) residues in position 2, and hydrophobic residues at the C-terminus, noted in published pool sequencing motifs for *B*3701*, *B*4402*, *B*4403*, B60 (*B*4001*), and B61 (*B*4006*) (DiBrino et al. 1995; Falk et al. 1993, 1995a; Harris et al. 1993; Fleischhauer et al. 1994; Thorpe and Travers 1994). One B18-restricted epitope (EBV 397–405, sequence DEVEFLGHY) has been reported in the literature (Steven et al. 1997), which suggests that the HLA molecules encoded by *B*18* alleles may also share this specificity. With the exceptions of *B*18* and *B*3701*, B44-supertype alleles possess K in position 45, suggesting that this positively charged residue is characteristic, and dictates the B pocket specificity, of B44-supertype alleles. Based on analysis of B and F pocket structures of various HLA alleles it is hypothesized that *B*4101*, *B*4501*, *B*4901*, and *B*5001* may also belong to the B44 supertype.

Biological validation, population coverage, and evolutionary significance

The studies referenced above demonstrate that a large fraction of HLA class I molecules can be classified into a relatively few supertypes, each characterized by largely overlapping peptide binding repertoires and consensus structures in the main peptide binding pockets. T-cell recognition data in infectious diseases and cancer contexts, obtained in a number of different studies, further demonstrate that cross-reactive peptides are frequently recognized in the course of natural disease processes (Bertoletti et al. 1997; Bertoni et al. 1997; Doolan et al. 1997; Fleischhauer et al. 1996; Kawashima et al. 1998; Khanna et al. 1998; Threlkeld et al. 1997; Wang et al. 1998) and in the context of multiple HLA molecules, underlining the biological significance of the cross-reactivities detected by HLA binding assays.

Taken individually, each of the HLA class I supertypes discussed above (A2, A3, B44, and B7) allows coverage of about 35–55% of the general population, irrespective of ethnicity (Sidney et al. 1996b). This might, in fact, represent a minimal estimate since it is based on analysis of only the most common HLA molecules, and population coverage might be expanded by other less common and as yet unidentified members of the various supertypes. However, when epitopes from the A2-, A3-, B7-, and B44-supertypes are combined, general population coverage in excess of 90% is achieved.

Interestingly, while the frequency of individuals positive for a given particular allele might vary drastically, the overall frequency of each supertype is remarkably constant across different ethnicities. For example in the case of the B7-supertype, B53 is present in 22.6% of Blacks, but only 0.2% of Japanese (Sidney et al. 1996b). Conversely B54 is found in 12.4% of the Japanese population, but is virtually absent in the Black population. However, the overall frequency of B7-supertype molecules is 54.7 in Blacks, and 55.1 in Japanese. Irrespective of the ethnicity considered, at least 43% of individuals express B7-like supertype molecules (Sidney et al. 1996b).

This high degree of overall expression, conserved amongst different populations, has potential biological implications and may be compatible with convergent evolution. It is also possible that the similar motifs grouped in a given supertype might be reflective of a common ancestry of the corresponding alleles. It is noteworthy that alleles of a given supertype are all encoded by either the HLA-A or the HLA-B locus, but not by both. This observation would tend to support the notion of common evolutionary origin. In this respect, since every main HLA-A evolutionary lineage contains at least one A3-supertype allele, and every HLA-B main evolutionary lineage contains at least one B7-supertype allele, A3- and B7-supertype molecules may document "primeval" HLA class I specificities.

Alternatively, convergent evolution might also explain these observations (Sidney et al. 1996a). It is notable that the main supertype specificities appear composed of "coupled" B- and F-pocket specificities. For example, a large fraction of alleles is associated with specificities for P in position 2 and hydrophobic residues at the C-terminus (B7-supertype), or with hydrophobic residues in position 2 and positive charges at the C-terminus (A3-supertype). Yet no motif has been reported which is composed of P in position 2 and positive charges at the C-terminus.

It is also interesting to note that human TAP molecules appear to be associated with peptide specificities largely overlapping with those of HLA supertypes. This observation suggests the hypothesis that the specifici-

ties of HLA molecules and the enzymatic machinery involved in antigen processing and presentation are subject to coordinate evolution (Sidney et al. 1996b; van Endert et al. 1996).

Can additional supertypes be defined?

The data described above summarizes the discovery and validation of four main HLA class I supertypes. Encouraged by these results, we undertook further analysis to examine whether additional supertypes might exist. For these studies, described herein for the first time, binding motifs were compiled either from data presented in the literature, or from our own studies. Additionally, we utilized the motif listings published by Rammensee and co-workers (1995, 1999). Binding motifs were defined using data from pool sequencing analyses, analysis of the binding capacity of large libraries of peptides, single substitution analysis, and sequence motifs frequently found in known epitopes. In certain cases, residues allowed within a motif were inferred on the basis of chemical similarity, or predicted on the basis of pocket analysis.

All HLA-A and -B alleles identified through 1995 (Parham et al. 1995) were included. In some cases, motifs have been reported for a specific allele, but not for other alleles corresponding to the same serological HLA antigen and with identical peptide binding pockets. In these instances, we have drawn the conclusion that highly related alleles of the same HLA antigen, which share identical peptide binding pockets, also share identical peptide binding motifs. To limit redundancy in the data, only one sequence was entered for alleles bearing the same serological antigen (i.e., subtypes) and identical HLA peptide binding pockets. The alleles sharing the same antigen and the same binding pockets have been noted in the tables.

For pocket analyses, the residues comprising the B and F pockets of HLA molecules described in crystallographic studies (Freemont et al. 1992; Guo et al. 1992; Madden et al. 1992, 1993; Matsumura et al. 1992; Saper et al. 1991) were compiled from the database of Parham and co-workers (1995). In these analyses, residues 9, 45, 63, 66, 67, 70, and 99 were considered to provide the peptide contact in the B pocket, and thereby to determine the specificity for the residue in the second position of peptide ligands. Similarly, residues 77, 80, 81, and 116 were considered to determine the specificity of the F pocket, and thereby the specificity for the C-terminal residue of a peptide ligand bound by the HLA molecule.

Population frequency data have been compiled from published sources (Fernandez-Viña et al. 1992; Imanishi et al. 1992; Krausa et al. 1995). Population frequency data is, in most cases, at the level of the HLA antigen, and only more rarely at the level of allelic subtypes. Total population coverage was calculated assuming Hardy-Weinberg equilibrium and considering only HLA molecules experimentally confirmed to share the supertype binding preference, and may therefore represent a minimal estimate. Furthermore, in cases where peptide binding, pool sequencing, or pocket structure analysis suggested that subtypes have similar peptide main-anchor preferences, a 1:1 correspondence between subtype alleles and the serologically defined antigens was assumed.

Definition of a potential HLA-A1-supertype

Evaluation of the pool sequencing motifs associated with the *A*0101*, *A*2601*, and *A*2602* alleles revealed important similarities. Specifically, these alleles bound peptides having a general motif of small (T, S) and hydrophobic (L, I, V, M, F) residues in position 2, and aromatic (Y, F, W) residues at the C-terminus (DiBrino et al. 1993b, 1994a; Dumrese et al. 1998; Kondo et al. 1997; Kubo et al. 1994). A similar motif has been inferred for two other alleles, *A*2501* and *A*3201*, based upon published sequences of known epitopes (Harrer et al. 1996; Rickinson and Moss 1997; van Baalen et al. 1996) (Tables 1, 2). These five alleles are tentatively defined as the A1-supertype. Considering residues tolerated by multiple A1-supertype alleles, an A1-supermotif may be defined as $(TSIVLM)_2$ and $(YWF)_c$.

Analysis of the B-pocket polymorphic residues of these five alleles revealed that they possessed a common pattern at the following positions: M_{45}, N_{66}, M or V_{67}, and H_{70}. In contrast, other alleles which are known to present a different specificity for position 2, such as *B*0702*, *B*2702*, *B*4402*, and *A*2402*, matched this consensus motif only partially, or not at all (Table 1). It is evident that the residues comprising the B pocket of the A1-supertype alleles are very similar to those of the alleles in the A2- and A3-supertypes, consistent with the observation that alleles of these three groups prefer aliphatic or hydrophobic residues in position 2 of their peptide ligands.

The F pocket of A1-supertype alleles is associated with two different structural motifs: N_{77}, T_{80}, L_{81}, D/N_{116} (*A*0101*, *A*2601*, and *A*2602*), and S_{77}, I_{80}, A_{81}, D_{116} (*A*2501* and *A*3201*) (Table 2). These two structural motifs are not found in any other HLA molecule associated with an F-pocket specificity differing from the A1-like specificity for aromatic residues.

Listed in Table 3 are the phenotypic frequencies of A1-supertype alleles in five major ethnic populations. The A1 supertype is represented with an average frequency of 25.2%, ranging from a low of 14.7% in Hispanics, to a high of 47.1% in Caucasians.

Examination of the sequences of HLA-A and -B alleles for which no peptide binding motif is known revealed that *A*0102*, *A*2604*, *A*3601*, *A*4301*, and *A*8001* possess A1-supertype B-pocket consensus sequences (Table 1), and also exactly match *A*0101* and *A*2601* in the F pocket (Table 2). On the basis of these

Table 1 B-pocket residues defining an HLA-A1-supertype

Antigen	Allele(s)	Position 2 motif	Reference(s)[a]	B-pocket residues[b]				
				9	45	66	67	70
A1	*A*0101*	TS	1–5	F	M	N	M	H
A25	*A*2501*	TIM	6–7	Y	M	N	V	H
A26	*A*2601*	VITLF	8	Y	M	N	V	H
A26	*A*2602*	VITLF	8	Y	M	N	V	H
A32	*A*3201*	I	9	F	M	N	V	H
A24	*A*2402*	YF	4, 10–11	S	M	K	V	H
B7	*B*0702*	P	10, 12–14	Y	E	I	Y	Q
B27	*B*2702*	R	15–16	H	E	I	C	K
B44	*B*4402*	E	17–19	Y	K	I	S	N
B57	*B*5701*	ATS	20	Y	M	N	M	S
B62	*B*1501*	QL	20–22	Y	M	I	S	N
A1	*A*0102*	Unknown		S	M	N	M	H
A26	*A*2604*	Unknown		Y	M	N	V	H
A36	*A*3601*	Unknown		F	M	N	M	H
A43	*A*4301*	Unknown		Y	M	N	V	H
A80	*A*8001*	Unknown		F	M	N	V	H

[a] References: 1. DiBrino et al. 1993b; 2. DiBrino et al. 1994a; 3. Kondo et al. 1997; 4. Kondo et al. 1994; 5. Falk et al. 1994; 6. Van Baalen et al. 1996; 7. Rickenson and Moss 1997; 8. Dumrese et al. 1998; 9. Harrer et al. 1996; 10. Maier et al. 1994; 11. Kondo et al. 1995; 12. Sidney et al. 1995; 13. Huczko et al. 1993; 14. Barber et al. 1995; 15. Rötzschke et al. 1994; 16. Jardetzky et al. 1991; 17. Fleischhauer et al. 1994; 18. Thorpe and Travers 1994; 19. DiBrino et al. 1995; 20. Barber et al. 1997; 21. Falk et al. 1995a; 22. Prilliman et al. 1997; 23. del Guercio et al. 1995; 24. Falk et al. 1991; 25. Ruppert et al. 1993; 26. Parker et al. 1992; 27. Sidney et al. 1997; 28 Sidney et al. 1996a; 29. diBrino et al. 1993a

[b] Residues hypothesized to form the B or F pocket, and to have potential contact with side chains of peptide residues. see, e.g., Saper and co-workers (1991) and Madden and co-workers (1992, 1993), Guo and co-workers (1992), and Freemont and co-workers (1992). Shaded residues indicates conformation with the putative supertype consensus pocket structure

Table 2 F-pocket residues defining an HLA-A1-supertype

Antigen	Allele(s)	C-terminus motif	Reference(s)[a]	F-pocket residues[b]			
				77	80	81	116
A1	*A*0101*	Y	1–5	N	T	L	D
A25	*A*2501*	W	6–7	S	I	A	D
A26	*A*2601*	YF	8	N	T	L	D
A26	*A*2602*	YF	8	N	T	L	N
A32	*A*3201*	W	9	S	I	A	D
A2	*A*0201*	LIVMAT	23–27	D	T	L	Y
A3	*A*0301*	RK	4, 10, 28–29	D	T	L	D
B7	*B*0702*	LIVMAFWY	10, 12–14	S	N	L	Y
A1	*A*0102*	Unknown		N	T	L	D
A26	*A*2604*	Unknown		N	T	L	D
A36	*A*3601*	Unknown		N	T	L	D
A43	*A*4301*	Unknown		N	T	L	D
A80	*A*8001*	Unknown		N	T	L	D

see Table 1 legend

shared structural features, *A*0102*, *A*2604*, *A*3601*, *A*4301*, and *A*8001* should therefore be included within the A1-supertype.

Definition of a potential HLA-A24-supertype

Peptides naturally bound by *A*2402* and *A*3001* HLA molecules (Kubo et al. 1994; Maier et al. 1994; Krausa and co-workers; as listed in Rammensee et al. 1999), are characterized by aromatic residues (F, W, Y) in position 2 and hydrophobic residues (Y, W, F, L, I, V, M) at the C-terminus (Tables 4, 5). This specificity has also been confirmed, in the case of *A*2402*, by extensive analysis of a peptide binding database (Kondo et al. 1995). Analysis of published epitopes recognized by *A*2301* (Khanna et al. 1996; Koziel et al. 1995) suggests that its peptide binding specificity might overlap with that of *A*2402* and *A*3001*. *A*2301* and *A*3001* also appear to accommodate small and/or hydrophobic resi-

Table 3 Phenotypic frequency of the HLA-A1-supertype

Antigen	Alleles	Subtypes in supertype		Phenotypic frequency (%)[a]					
		Defined	Predicted	Caucasian	NA Black	Japanese	Chinese	Hispanic	Average
A1	*A*0101-02*	*A*0101*	*A*0102*	28.6	10.1	1.4	9.2	10.1	11.9
A25	*A*2501*	*A*2501*	–	6.1	1.6	0.0	0.8	4.2	2.5
A26	*A*2601-04*	*A*2601-02*	*A*2604*	7.3	3.2	20.4	3.8	6.7	3.9
A32	*A*3201*	*A*3201*	–	9.6	1.6	0.2	1.2	6.7	3.9
A36	*A*3601*	–	*A*3601*	0.8	5.3	0.0	0.4	0.6	1.4
A43	*A*4301*	–	*A*4301*	0.0	0.2	0.2	0.0	0.0	0.1
A80	*A*8001*	–	*A*8001*	–	–	–	–	–	–
Total coverage				47.1	16.1	21.8	14.7	26.3	25.2

[a] Total coverage assumes Hardy-Weinberg equilibrium. The total coverage is calculated considering only those antigens or alleles experimentally confirmed to share the supertype binding preference, and therefore represents a minimal estimate. Where peptide-binding data, pool-sequencing analysis, or pocket structure based on primary sequence suggest that subtypes will have very similar, if not identical, peptide main-anchor preferences and overlapping peptide-binding specificities, a 1:1 correspondence between subtype alleles and the serologically defined antigens was assumed. As peptide binding motifs for more alleles are reported, it is conceivable that the population coverage achieved by a particular supertype will increase

Table 4 B-pocket residues defining an HLA-A24-supertype

Antigen	Allele(s)	Position 2 motif	Reference(s)[a]	B-pocket residues[b]				
				9	45	66	67	70
A23	*A*2301*	IY	1–2	S	M	K	V	H
A24	*A*2402*	YFW	3–5	S	M	K	V	H
A30	*A*3001*	YFVLMIT	6	S	M	N	V	Q
A26	*A*2601*	VITLF	7	Y	M	V	H	H
A31	*A*3101*	LIVMAST	8–9	T	M	N	V	H
B27	*B*2702*	R	10–11	H	E	I	C	K
B44	*B*4402*	E	12–14	Y	K	I	S	N
B57	*B*5701*	ATS	15	Y	M	N	M	S
B62	*B*1501*	QL	15–17	Y	M	I	S	N
B7	*B*0702*	P	4, 18–20	Y	E	I	Y	Q
A24	*A*2403*	Unknown		S	M	K	V	H
A24	*A*2404*	Unknown		S	M	K	V	H
A30	*A*3002-03*	Unknown		S	M	N	V	H

[a] References: 1. Koziel et al. 1995; 2. Khanna et al. 1996; 3. Kubo et al. 1994; 4. Maier et al. 1994; 5. Kondo et al. 1995; 6. Krausa et al. submitted; 7. Dumrese et al. 1998; 8. Falk et al. 1994; 9. Sidney et al. 1996a; 10. Rötzschke et al. 1994; 11. Jardetzky et al. 1991; 12. Fleischhauer et al. 1994; 13. Thorpe and Travers 1994; 14. DiBrino et al. 1995; 15. Barber et al. 1997; 16. Falk et al. 1995a; 17. Prilliman et al. 1997; 18. Sidney et al. 1995; 19. Huczko et al. 1993; 20. Barber et al. 1995; 21. del Guercio et al. 1995; 22. Falk et al. 1991; 23. Ruppert et al. 1993; 24. Parker et al. 1992; 25. Sidney et al. 1997; 26. DiBrino et al. 1993a

[b] Residues hypothesized to form the B or F pocket, and to have potential contact with side chains of peptide residues. See, e.g., Saper and co-workers (1991) and Madden and co-workers (1992, 1993), Guo and co-workers (1992) and Freemont and co-workers (1992). Shaded residues indicates conformation with the putative supertype consensus pocket structure

Table 5 F-pocket residues defining an HLA-A24-supertype

Antigen	Allele(s)	C-terminus motif	Reference(s)[a]	F-pocket residues[b]			
				77	80	81	116
A23	*A*2301*	WI	1–2	N	I	A	Y
A24	*A*2402*	FLI	3–5	N	I	A	Y
A30	*A*3001*	LYFM	6	D	T	L	H
A2	*A*0201*	LIVMAT	21–25	D	T	L	Y
A3	*A*0301*	RK	3–4, 8, 26	D	T	L	D
B7	*B*0702*	LIVMAFWY	4, 18–20	S	N	L	Y
A24	*A*2403*	Unknown		N	I	A	Y
A24	*A*2404*	Unknown		N	T	L	Y
A30	*A*3002-03*	Unknown		N	T	L	H

See Table 4 legend

Table 6 Phenotypic frequency of the HLA-A24-supertype

Antigen	Alleles	Subtypes in supertype		Phenotypic frequency (%)[a]					
		Defined	Predicted	Caucasian	NA Black	Japanese	Chinese	Hispanic	Average
A23	*A*2301*	*A*2301*	–	3.2	14.3	0.0	1.6	5.5	4.9
A24	*A*2402-04*	*A*2402*	*A*2403-04*	16.8	8.8	58.1	32.9	26.7	28.7
A30	*A*3001-03*	*A*3001*	*A*3002-03*	4.7	18.8	0.8	7.3	8.4	8.0
Total coverage				23.9	38.9	58.6	40.1	38.3	40.0

[a] See Table 3 legend

dues (L, V, I, M, T) in position 2. Thus, an A24-supermotif incorporating residues commonly recognized by A24-supertype alleles may be defined as $(FWYL-VIMT)_2$ and $(FIYWLM)_c$.

*A*2301* and *A*2402* share an identical B-pocket structural motif of S_9, M_{45}, E_{63}, K_{66}, V_{67}, H_{70}. The B-pocket structure of *A*3001* differs from that of *A*2301* and *A*2402* at 3 positions: F_9, N_{66}, and Q_{70}. Neither of these structural motifs are found in alleles with different B-pocket specificities (Table 4).

The presence of a small residue (S) in position 9, as opposed to the F or Y present in most other HLA-A alleles, is hypothesized to allow *A*2301* and *A*2402* to accommodate large aromatic residues in position 2 of their peptide ligands. Furthermore, it is noted that the *A*3001* B pocket matches that of *A*0301*, which accommodates a broad range of residues in position 2 of its peptide ligands (Kubo et al. 1994; Sidney et al. 1996a).

The following F-pocket residues of *A*2301* and *A*2402* are identical: N_{77}, I_{80}, A_{81}, Y_{116} (Table 5). This motif is also found in HLA-B alleles (*B*5101-05*, *B*5201*, and *B*5702*), which share the same C-terminal, but not the same B-pocket, specificity (Barber et al. 1997; Falk et al. 1995b; Sidney et al. 1996b). The F pocket structure of *A*3001* (D_{77}, T_{80}, L_{81}, H_{116}) is again somewhat different from that of *A*2301* and *A*2402*, and is indeed unique among HLA alleles; no other alleles sequenced to date possess H in position 116.

Of alleles for which no peptide binding motif is known, only the *A*24* and *A*30* subtypes *A*2403*, *A*2404*, *A*3002*, and *A*3003* possess B and F pocket structures identical, or conservatively similar, to the A24-supertype alleles. These alleles have also been tentatively included within the A24-supertype. The A24-supertype is represented in the five major ethnic populations with an average frequency of 40.0%, ranging from a low of 23.9% in Caucasians, to a high of 58.6% in Japanese (Table 6).

Additional potential HLA-B-supertypes

Pool sequencing motifs for peptides bound to *HLA-B*1401-02*, *B*1503*, *B*1509*, *B*1510*, *B*1518*, *B*3801-02*, *B*3901*, *B*3902*, *B*3903-04*, *B*4801-02*, *B*7301*, and *B*2701-08* (Barber et al. 1996a, 1997; Boisgérault et al. 1996; DiBrino et al. 1994b; Falk et al. 1995c; Rötzschke et al. 1994; Garcia et al. 1997; Jardetzky et al. 1991) share positively charged (R, H, K) residues in position 2, and hydrophobic (A, L, I, V, M, Y, F, W) residues at the C-terminus (Tables 7A, B). These alleles, because of their shared overlapping peptide specificity, have been collectively designated as the B27-supertype.

Analysis of the relevant B-pocket residues of these alleles reveals a B27-supertype consensus structural motif of E in position 45, and either C or S in position 67. An E in position 45 is also present in some B7-supertype alleles, but only in combination with F or Y in position 67. Similarly, S in position 67 is seen also in B44- and B62-supertype alleles (see below), but never in combination with E in position 45. C in position 67 appears to be exclusive to B27-supertype alleles.

*B*1503*, *B*1510*, *B*1518*, *B*2701*, *B*2708*, *B*4801*, and *B*4802* also possess the B27-supertype B pocket consensus structural motif. The structure of their F pockets is consistent with those of other HLA-B alleles binding peptides with hydrophobic C-termini. These alleles have also been tentatively included within the B27-supertype. The B27-supertype is represented with an average frequency of 23.4% in five major ethnic populations. The frequency of the supertype ranges from a low 13.3% in Japanese, to a high of 35.3% in Hispanics.

By contrast, *B*1516*, *B*1517*, *B*5701*, *B*5702*, and *B*58* share a preference for small aliphatic residues (A, S, and T) in position 2, and aromatic (F, W, Y) or hydrophobic (L, I, V) residues at the C-terminus of their peptide ligands (Barber et al. 1997; Falk et al.1995a). The B pocket structures of these alleles, collectively designated the B58-supertype, indicate a consensus motif of Y_9, N_{66}, M_{67}, S_{70}. This structural motif is entirely unique to B58-supertype alleles. The B58-supertype is represented in the Black population with a frequency of 25.1%. Overall, it is represented, on average, in the five major ethnic populations with a frequency of 10.3%.

Finally, the pool sequencing motifs of *B*4601*, *B*52*, *B*1501* (*B62*), *B*1502* (*B75*), and *B*1513* (*B77*) reveal a shared preference for ligands with the polar aliphatic residue Q, or hydrophobic aliphatic residues (L, V, M, P and I), in position 2, and hydrophobic residues (F, W, Y, M, I, V) at the C-terminus (Barber et al. 1996, 1997;

Table 7 Summary of HLA-supertypes
A Overall phenotypic frequencies of HLA-supertypes in different ethnic populations

Supertype	Specificity[a]		Phenotypic frequency					
	Position 2	C-terminus	Caucasian	N.A. Black	Japanese	Chinese	Hispanic	Average
B7	P	AILMVFWY	43.2	55.1	57.1	43.0	49.3	49.5
A3	AILMVST	RK	37.5	42.1	45.8	52.7	43.1	44.2
A2	AILMVT	AILMVT	45.8	39.0	42.4	45.9	43.0	42.2
A24	YF [WIVLMT]	FI [YWLM]	23.9	38.9	58.6	40.1	38.3	40.0
B44	E [D]	FWYLIMVA	43.0	21.2	42.9	39.1	39.0	37.0
A1	TI [LVMS]	FWY	47.1	16.1	21.8	14.7	26.3	25.2
B27	RHK	FYL [WMI]	28.4	26.1	13.3	13.9	35.3	23.4
B62	QL [IVMP]	FWY [MIV]	12.6	4.8	36.5	25.4	11.1	18.1
B58	ATS	FWY [LIV]	10.0	25.1	1.6	9.0	5.9	10.3

B Estimated population coverage afforded by different HLA-supertype combinations

HLA-supertypes	Phenotypic frequency					
	Caucasian	N.A. Black	Japanese	Chinese	Hispanic	Average
A2, A3, and B7	83.0	86.1	87.5	88.4	86.3	86.2
A2, A3, B7, A24, B44 and A1	99.5	98.1	100.0	99.5	99.4	99.3
A2, A3, B7, A24, B44, A1, B27, B62, and B58	99.9	99.6	100.0	99.8	99.9	99.8

[a] Motifs indicate the residues defining supertype specificities. The motifs incorporate residues determined on the basis of published data to be recognized by multiple alleles within the supertype. Residues within brackets are additional residues also predicted to be tolerated by multiple alleles within the putative supertype

Falk et al. 1995a, b; Prilliman et al. 1997). On the basis of this shared specificity, these alleles have been designated the B62-supertype.

B62-supertype alleles carry a B-pocket consensus motif of Y_9, M/T_{45}, I_{66}, S_{67}, N_{70}. This structural motif is similar to that found in some B44- and B27-supertype alleles, except that B62-supertype alleles do not possess a charged residue at position 45. *B*1301-02*, *B*1506*, *B*1512*, *B*1514*, *B*1519*, and *B*1521* are additional HLA-B alleles which match the B62-supertype B pocket consensus structural motif, and which have F pocket structures consistent with a hydrophobic specificity. Thus, these alleles have been tentatively included within this supertype.

The B62-supertype is represented with an average frequency of 18.1%, ranging from a low of 4.8% in Blacks to a high of 36.5% in Japanese. It should be noted that the B62-supermotif has similarities to the A2- and A24-supermotifs. Thus, additional coverage may be achieved by including some or all of these alleles in the A2- and/or A24-supertypes.

Major histocompatibility complex polymorphism and military corps: an analogy

Together, the previous and present analyses indicate that a total of nine HLA-supertypes may exist. Table 7A summarizes these supertypes, and indicates an estimate of their prevalence in major ethnic groups.

It appears that all known HLA class I A and B alleles might be classified in one of these nine supertypes (A29, B8, and B46 being exceptions). We currently estimate that six major supertypes alone account for over 85% of HLA-A genes, and 40% of HLA-B genes. In practical terms, epitopes from just the six most frequent supertypes afford an average population coverage of 99.3% (98.1 to 100% for five major ethnic groups; see Table 7B). As mentioned above, the overall frequency of each of these supertypes is remarkably high and fairly conserved among very different ethnicities. Thus, there might be some advantage for human populations to present approximately five to ten main binding specificities, and that each one of these is maintained at relatively high frequency.

What is the biological meaning of these observations? A book of old military uniforms might provide a useful analogy. At first glance, a similarly high, and hard to comprehend, degree of diversity is noted (Heck 1994). One might wonder about the significance of so many different types of military corps and uniforms and how to classify them. Analysis of their function will, however, reveal that most military corps can in fact be classified into a few broad categories: "navy", "artillery", "cavalry", "infantry", and so on. In each army, irrespective of nationality, uniforms belonging to each category are represented in relatively high and relatively constant frequencies. It would be difficult to find an army made of artillery only, with no cavalry, infantry or navy.

To fight a war requires a balance amongst different military functions. No function is all-encompassing, and an army which is not diversified might be eliminated. Yet there are a limited number of effective army func-

tions, and an army lacking a crucial function will likely be doomed. Recent military history illustrates this point. Wars are not won by air or sea supremacy alone, and by the same token, an opponent severely outdone in terms of air support can be in serious trouble.

In this respect, different HLA alleles are analogous to different military corps, and whole armies to populations fighting disease. Main supertypes would then correspond to main military functions.

The collective immune system of human populations is at constant war with pathogens. HLA supertypes describe the main functional specificities of peptide class I binding. Each population in its own way, as an adaptation to local conditions, will fine-tune its main population's peptide binding specificities, and optimize it's capacity to fight the war against pathogens.

Conclusion

The data described above illustrates how the vast majority of known HLA class I binding specificities can be classified into nine major functional supertypes. The relevance of this observation might well expand beyond the human species, as it has recently been noted that HLA-supertype specificities extend to chimpanzees (Bertoni et al. 1998; Kowalski et al. 1996), macaques (Allen et al. 1998), and gorillas (Urvakes and co-workers, unpublished data). Independent observations demonstrate that the motif of the mouse class I allele L^d is identical to the B7-supermotif (Corr et al. 1992; Falk et al. 1991)

While the necessary task of fully exploring and cataloging MHC polymorphism continues, we have begun to appreciate the common denominators and similarities hidden within this very large degree of polymorphism. A classification based on MHC function (which is, after all, to bind peptides), is possible. This classification can help to illustrate how the many different populations and ethnic backgrounds which make up our human species are engaged in a never-ending war against disease, and must outsmart pathogens having a capacity to mutate much faster than MHC alleles. We are utilizing this knowledge as a tool to design effective vaccines for the treatment and prevention of human diseases.

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