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Evolution of HLA Antigenic Determinants: Species Cross-reactions of Monoclonal Antibodies

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Abstract. The reactions of mouse monoclonal HLA-A-, -B-, -C-specific antibodies on cells from 50 species were analyzed by binding assay. Antibodies which are monomorphic in humans generally show monomorphic behavior in other species and many significant cross-reactions were seen. Antibodies which recognize highly specific polymorphic determinants gave few cross-reactions with other species. Antibodies which recognize broad polymorphic determinants in humans cross-reacted in a polymorphic manner with many other species. The patterns of cross-reaction for both monomorphic and broadly polymorphic determinants correlated roughly with the current picture of phylogenetic relationships. These results show there are two fundamentally different classes of polymorphic determinants, one which appears relatively conserved in evolution and one which is the product of recent change. They probably correlate with the public and private antigens of mouse H-2 antigens. A simple genetic model of *HLA-A, -B, -C* genes, whereby all specificities at a locus are true alleles, is sufficient to explain these patterns of cross-reaction. It seems likely that cross-reactions previously seen with alloantisera were due to broadly polymorphic antibodies rather than to allele-specific antibodies. If this were the case, then no experimental basis for Bodmer's model of MHC polymorphism being due to control of expression of a tandem array of genes exists.

Introduction

A major histocompatibility system has been demonstrated in all mammalian, avian, and several fish and amphibian species studied (Götze 1977). Biochemical

Abbreviations used in this paper: MHC = major histocompatibility complex; PBS = phosphate-buffered saline; BSA = bovine serum albumin; RAM = rabbit anti-mouse IgG $F(ab')_2$ fragments.

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characterization of the histocompatibility antigens revealed their basic molecular structure to be the same in every species so far examined. Another important characteristic of these antigens is their high polymorphism within a species, which has allowed an extensive analysis of their properties to be made using alloantisera (Klein 1975, Snell et al. 1976, Svejgaard et al. 1979). When alloantisera were first tested against evolutionarily distant species a considerable amount of cross-reactivity was observed (Albert et al. 1969, Abeyounis et al. 1969, Ivasková et al. 1972, Iha et al. 1973). Many investigators have subsequently studied the serological cross-reactions between the histocompatibility antigens of various species and obtained similar results. This work has recently been reviewed by Ivanyi (1981). The finding that alloantisera cross-react with distantly related species suggested that the histocompatibility polymorphisms were in fact conserved during evolution. To explain such an unexpected phenomenon Bodmer (1973) proposed that histocompatibility antigen polymorphism is generated at the level of antigen expression and that every member of a species has the information to code for the histocompatibility antigens of the entire species in its genome. Thus, the complete set of polymorphisms could be inherited as a group and evolve at the rate of any other piece of genetic material. If these polymorphisms arose from gene duplications which occurred prior to speciation then it is conceivable that two different species might express the same polymorphisms.

Studies demonstrating the appearance of "alien" H-2 antigens on transformed cells claim to support this model of polymorphism for control of gene expression, since they suggest that certain cells can be induced to express nonself histocompatibility antigens (Parmiani et al. 1979, Pellegrino et al. 1976).

Recently monoclonal antibodies to histocompatibility antigens have become available and preliminary studies indicated that some cross-reacted significantly with cells of other species (Brodsky et al. 1979b, McMaster et al. 1979, Gasser et al. 1979, Smilek et al. 1980, Boyd et al. 1981, Russo et al. 1980). In the process of characterizing monoclonal anti-HLA reagents it became apparent that analysis of the cross-reactions on nonhuman species was often useful for distinguishing antibodies which were identical in their reactions with human cells (Brodsky et al. 1979b). This type of study also provided an opportunity to follow the phylogenetic distribution of individual antigenic determinants and reassess the evolution of polymorphic HLA determinants. In this paper the results of testing a panel of 29 monoclonal HLA* reagents against 50 different species are discussed.

Materials and Methods

Cell lines and tissue culture. B lymphoblastoid cell lines Mich, IDF, WT20, PGF, WT46, Daudi (from the Genetics Laboratory, Oxford, U. K.), JY, LB (a gift of J. Strominger), B17B (a gift of W. Biddison), Pala (a gift of P. Cresswell), and CYA (produced in our laboratory from peripheral blood lymphocytes kindly provided by R. Payne) were grown in RPMI 1640 medium supplemented with 10% horse serum or fetal calf serum (FCS), penicillin (100 units/ml), and streptomycin (100 units/ml) (basic medium).

* The terms HLA antibody or HLA antibodies (reagents) will refer to mouse monoclonal antibodies directed against HLA-, -B, -C molecules.

Monoclonal antibodies. All monoclonal antibody reagents were prepared either from supernatants of hybridoma cell lines or from ascites fluids of animals carrying the hybridoma cell line as a tumor. Hybridoma cell lines were grown in RPMI 1640 medium supplemented with 10% FCS and 10^{-4} M hypoxanthine, 1.6×10^{-5} M thymidine, and 4×10^{-7} M aminopterin. The W6/32 hybridoma was a gift of C. Milstein and the A28M[1] hybridoma a gift of F. C. Grumet. References describing the production and characterization of all antibodies used are listed in Table 1.

Blood collection and lymphocyte separation. Blood was collected in heparin, Alsever's solution or acid-citrate-dextrose and lymphocytes separated as described (Boyum 1968, Amorena and Stone 1980). Lymphocytes from the hyrax, toad, and axolotl were obtained by dissection of spleens in PBS, pH 7.4. Mouse lymphocytes were obtained by dissection of thymus in PBS.

Radioactive binding assay. Binding assays were based on the method of Williams (1977) as described previously (Brodsky et al. 1979a, b). Antibodies were used at the concentration found in hybridoma culture supernatant (10 μg–30 μg/ml).

Results

Assay for antibody cross-reactivity

An indirect trace radioimmunoassay was used to detect antibody binding to lymphocytes of all species (Williams 1977). ^{125}I-labeled RAM (^{125}I-RAM) was used as the second-step reagent. This assay was chosen because many antibodies tested were of the IgG isotype and less than 1/5 were cytotoxic in a complement-dependent assay. Furthermore, most of the antibodies tested were initially selected and characterized by indirect trace radioimmunoassay on human B lymphoblastoid cell lines so their behavior in this system was known. This assay, which includes four washes to remove unbound antibody and five washes to remove unbound ^{125}I-RAM, requires a minimum avidity of approximately 10^7 M^{-1} for an antibody cross-reaction to be detected and does not discriminate between high levels of binding because it is a trace assay. However, since all cells were tested under the same conditions, a comparison between tests provides a valid comparison between antibodies and the determinants they detect. The following criteria were established to characterize the cross-reactions observed for the HLA-specific antibodies. Antibody reactions no greater than twice the background binding with assay buffer (0.5% BSA-PBS) were considered negative. Reactions three to four times the background binding were considered marginal and those reactions five times the background binding or more were classified as positive. The binding of these antibodies to human cells varied from ten to 40 times the background depending on the antibodies being tested.

Specificity of the HLA-specific monoclonal antibodies tested for cross-reactivity

Table 1 shows the 29 HLA-specific monoclonal antibodies used in this study. Analysis by a variety of biochemical and serological procedures showed that the determinants or epitopes recognized by the HLA antibodies divide into three distinct groups* (Brodsky and Parham 1981).

* For convenience antibodies in these three groups will be referred to as monomorphic, broadly polymorphic or specific polymorphic antibodies.

Table 1. Monoclonal HLA-A, -B-, -C-, DR-specific antibodies tested for cross-reactivity with nonhuman species

Antibody	Specificity	Group	Reference
PA2.2	No binding to Aw32, B13*	I[†]	Parham and Bodmer 1978
PA2.5	Broadly polymorphic[‡]	I	Parham and Bodmer 1978
BB7.6	No binding to A2, A28, A3, A25, B12, B27	I	Brodsky et al. 1979b
MB40.1	No binding to A1, A3, A9, B12, B14, B27, Bw35, B37	I	Parham and McLean 1980
MB40.4	No binding to A1, A2, Bw35, B44, B62	I	Parham 1981
MCLB.1	No binding to A2	I	
MCLB.2	No binding to A2	I	
PB40.1	No binding to A2, A3, A30, B15, B18, B27, Bw35	I	
KA28.1	No binding to A2, A3, A28, A30, B7, B15, B18, Bw35, B39, B27	I	
W6/32	Monomorphic A, B, C[‖]	II[§]	Barnstable et al. 1978
MB40.5	Monomorphic A, B, C	II	Parham 1981
PA2.6	Monomorphic A, B, C	II	Parham and Bodmer 1978
BBM.1	β_2-microglobulin	II	Brodsky et al. 1979a
PA2.12	β_2-microglobulin	II	Parham and Bodmer 1978
BB7.3	β_2-microglobulin	II	Brodsky et al. 1979b
BB7.4	β_2-microglobulin	II	Brodsky et al. 1979b
MCLB.3	β_2-microglobulin	II	
MCLB.4	β_2-microglobulin	II	
BB7.5	A, B, C-β_2m complex[¶]	II	Brodsky et al. 1979b
BB7.7	A, B, C-β_2m complex	II	Brodsky et al. 1979b
BB7.8	A, B, C-β_2m complex	II	Brodsky et al. 1979b
BB7.9	A, B, C-β_2m complex	II	Brodsky et al. 1979b
PA2.1	Binds only to A2 and A28*[△]	III[▽]	Parham and Bodmer 1978
BB7.1	Binds only to B7	III	Brodsky et al. 1979b
BB7.2	Binds only to A2 and A28*	III	Brodsky et al. 1979b
MA2.1	Binds only to A2 and B17	III	McMichael et al. 1980
MB40.2	Binds only to B7 and B40	III	Parham 1981
MB40.3	Binds only to B7 and B40	III	Parham 1981
A28M[1]	Binds only to A2, A28, A28*[○]	III	Gift of F. C. Grumet

* The specificity of broad polymorphic antibodies has been defined by identifying the HLA-A and B antigens with which they do not react.

[†] Group I contains all the broadly polymorphic HLA-A, -B-specific monoclonal antibodies, as described in the text.

[‡] No completely negative reactions have been seen with PA2.5. Evidence for polymorphism is from cross-reactivity in other species, as will be discussed.

[‖] Monomorphic A, B, C refers to monomorphic reactivity with the HLA heavy chain of the A, B, and C antigens.

[§] Group II contains all the monomorphic HLA-A, -B, -C-specific monoclonal antibodies, as described in the text.

[¶] These antibodies recognize a determinant dependent on the presence of both HLA heavy chain and β_2m for its antigenicity.

[△] A28* is a variant of A28 recognized by the PA2.1 and BB7.2 antibodies. (Parham and Brodsky 1981)

[▽] Group III contains all the specific polymorphic HLA-A, -B-specific monoclonal antibodies, as described in the text.

[○] KMO1 binds to A2 and both the normal and variant A28. (F. C. Grumet personal communication, J. P. Ways unpublished results)

Group I: Broadly polymorphic antibodies. These antibodies react with many different HLA-A, -B, -C molecules, invariably with products of more than one locus, and show differential avidity within the group of positively reacting molecules. An example is the MB40.1 antibody which reacts strongly with A28, Aw32, B7, B8, and B40, shows no reaction with A1, A3, A9, B12, B14, B27, Bw35, and B37, and has a spectrum of intermediate reactions with other HLA-gene products (Parham and McLean 1980). This type of complex specificity has rarely been analyzed in *HLA* serology, notable exceptions being the Bw4 and Bw6 determinants (van Rood et al. 1970). Such determinants are probably analogous to the public H-2 antigens of the mouse (Klein 1979).

Group II: Monomorphic antibodies. Monomorphic antibodies recognize determinants common to all HLA molecules, which they bind with similar avidity. A minimum of four such determinants, one on β_2-microglobulin (β_2m), one on the HLA heavy chain, and two combinatorial determinants formed by both chains have been defined (Brodsky and Parham 1981). Monomorphic determinants represent structures which are conserved within a species but must differ between species otherwise antibodies against them could not be so readily produced. Analogous determinants for H-2 antigens have not yet been described.

Group III: Specific polymorphic antibodies. These antibodies react with highly specific polymorphic determinants present on only one or two different HLA molecules. Examples are the B7-specific determinant recognized by the BB7.1 antibody, the determinant shared by A2 and B17 recognized by the MA2.1 antibody, and the determinant shared by A2 and a variant of A28 recognized by the BB7.2 and PA2.1 antibodies (Brodsky et al. 1979, McMichael et al. 1980).Their specificity approximates that of specific HLA typing alloantisera and the determinants they identify are analogous to the private H-2 antigens of the mouse (Klein 1979).

Figure 1 shows an example of the binding activity of one monoclonal antibody from each of these categories to a panel of B lymphoblastoid cell lines of different HLA-A, -B, -C types. It can be seen that BB7.2 antibody bound only to lines expressing A2 and A28* (variant) and not to lines expressing A28 (common) and other specificities. MB40.1 bound to all cell lines with a large variation in avidity. W6/32 bound similarly to all cell lines.

Cross-reactivity of HLA-specific monoclonal antibodies with lymphocytes from nonhuman species

The 29 antibodies shown in Table 1 were tested for binding to lymphocytes from various individuals of 50 species by indirect trace radioimmunoassay. The results are summarized in Tables 2, 3, and 4. It became apparent that the three groups of HLA antibodies, defined previously on the basis of reactions with human cells, behaved as distinct groups with regard to their patterns of cross-reaction. We have therefore presented the results separately with broadly polymorphic antibodies in Table 2, monomorphic antibodies in Table 3, and specific polymorphic antibodies in Table 4, and will discuss each group in turn.

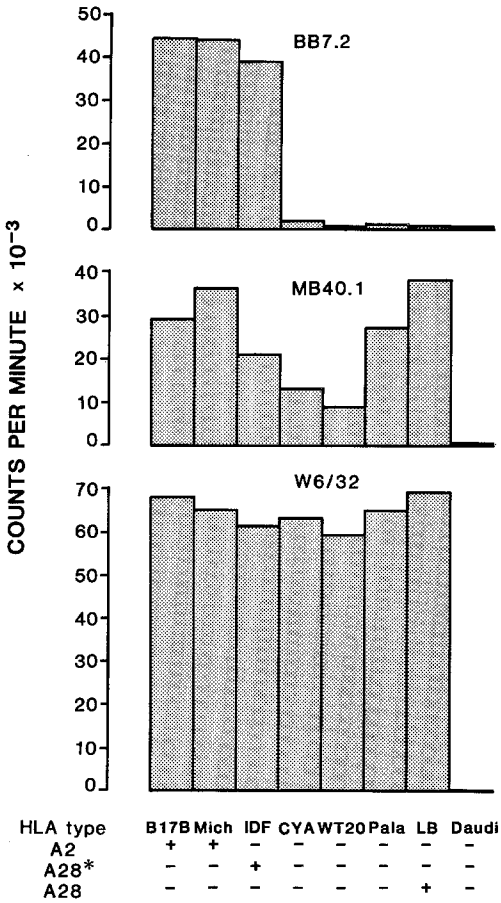

Fig. 1. Binding of a specific polymorphic (BB7.2), broadly polymorphic (MB40.1), and monomorphic (W6/32) HLA monoclonal antibody to B lymphoblastoid cell lines. Monoclonal antibodies in hybridoma supernatant form were tested for reactivity with B lymphoblastoid cell lines (2×10^5 target cells) by indirect trace binding assay. Counts bound shown are the mean of duplicate assays. The HLA-A, -B types of the cell lines used are B17B (A1, 2; B7, 8), Mich (A2, 32; B15, 27), IDF (A26, 28*; B18, 38), CYA (A11, 26; Bw22, w51), WT20 (A30, 30; B18, 18), Pala (A9, 9; B8, 8), LB (A28, 28; B40, 40). Daudi expresses no HLA-A, -B, -C antigens (Nilsson et al. 1974).

Broadly polymorphic antibodies. Table 2 shows the results of testing the broadly polymorphic antibodies against lymphocytes of 50 different species for indirect trace binding activity. Two characteristic features of the properties of broadly polymorphic antibodies can be discerned from the results shown in Table 2. First, most of the antibodies in this group (seven out of nine) showed binding to cells of many species and, second, their cross-reactivity was found to be polymorphic when a large enough group of individuals from a given species was tested. For example, the MB40.1 antibody, which bound to 35 out of 41 human cell lines tested, bound cells of six out of 11 ring-tailed lemurs tested. As a rough generalization, it can be seen that there are a greater number of cross-reactions with species considered by other criteria to be more closely related to humans. This is illustrated by the cross-reactions of PA2.2, PA2.5, BB7.6, and MB40.1 which were tested against both apes and prosimians. As a group, these antibodies gave positive reactions with cells of 41 out of 47 apes* tested (87 percent) and with cells of only 55 out of 113 prosimians†

* Apes include the gorilla, chimpanzee, gibbon, and orangutang.
† Prosimians include the lemur, galago, potto, and slow loris.

tested (49 percent). However, for any given determinant there is not a predictable pattern of reactivity which corresponds with evolutionary distance between species. This is most clearly seen for groups of related species such as the lemurs, where an antigenic determinant can often be found in one species and not in another, e. g., PA2.5 bound to cells of two out of three black lemurs but showed no binding to cells of any of the 14 ring-tailed lemurs tested. Another rough generalization that can be made is that the frequency of antibody cross-reactions within a species (representing the phenotypic frequency of the determinants) has a tendency to decrease with the degree of evolutionary separation of these species from humans. Thus, the PA2.2 antibody reacted with all human cells tested except for those from one individual (more than 99 percent), two out of four rhesus monkeys (50 percent), and four out of 12 ring-tailed lemurs (33 percent). Binding of the PA2.5 antibody provided a more extreme example of this phenomenon. This antibody reacted with all human cells tested, appeared monomorphic in apes and old-world monkeys but displayed polymorphic behavior in owl monkeys and spider monkeys, binding to cells of 42 out of 52 animals tested (80 percent). PA2.5 showed a further decrease in phenotypic frequency in tree shrews where it bound to cells of two out of five animals tested (40 percent). This pattern of species cross-reaction revealed that the PA2.5 antibody was polymorphic, which was not obvious from its binding behavior with human cells (Parham et al. 1979a).

Monomorphic antibodies. When the results with broadly polymorphic antibodies are compared with those obtained with monomorphic antibodies, shown in Table 3, there is a striking difference. Five out of the 13 monomorphic antibodies show extensive cross-reactions in other species but the reactions seen were always monomorphic, i. e., the reactions with every individual within a species, whether positive or negative, were the same. It is apparent that the degree of confidence with which one can make this statement for any species depends upon the sample size, which varied considerably. However, there have been no exceptions to this hypothesis. One interesting and special case is the owl monkey. Different races or subspecies of owl monkeys have been identified on the basis of coat color, geographical location, and karyotypic differences. These have been classified according to karyotype by Ma and co-workers (1976). Antibodies such as W6/32, which recognize the monomorphic determinant of the HLA heavy chain react with owl monkeys of karyotypes VI and VII, but not with those of karyotypes I, II, III, IV, and V. Thus, monomorphic behavior is essentially observed for these antibodies in owl monkeys but is complicated by the exact and debated definition of what constitutes a species (Parham et al. 1979a, b).

All 13 monomorphic antibodies tested gave a significant number of strong cross-reactions with cells of other species and, as observed for broadly polymorphic antibodies, cross-reactions are more likely to be found with species which are evolutionarily closer to humans. It is also possible to trace the evolutionary inheritance of the different monomorphic determinants. Thus, the BBM.1 β_2-m determinant was found only in African apes, the BB7.5 combinatorial determinant in African and Asian apes, W6/32 in apes and old-world monkeys, and the BB7.7 combinatorial determinant in apes and most monkeys. The more conserved determinants also showed some reactions among other groups of species. W6/32

Table 2. Binding of broadly polymorphic HLA-A, -B-, -C-specific antibodies to peripheral blood cells of nonhuman species

Species	Antibody								
	PA2.2	PA2.5	BB7.6	MB40.1	MB40.4	MCLB.1	MCLB.2	PB40.1	KA28.1
Gorilla (*Gorilla gorilla*)		4/4	3/3	0/2	0/3	2/2	2/2		
Chimpanzee (*Pan troglodytes*)	8/8	12/12	4/4	3/4	1/9	1/9	4/9	4/4	
Gibbon (*Hylobates lar*)		7/7			0/7				
Orangutang (*Pongo pygmaeus*)*	0/1	0/1	0/1						
Baboon (*Papio anubis*)	3/4	4/4	0/2	1/1	0/1	1/1	1/1	1/1	0/1
Crab- eating macaque (*Macaca fascicularis*)†	0/1	1/1	0/1	M1/1	0/1	1/1	1/1	1/1	0/1
Blue monkey (*Cercopithicus mitis*)		4/4							
Rhesus monkey (*Macaca mulatta*)	2/4	16/16	1/2	M1/11	0/11	6/6	6/6	M1/1	0/1
Owl monkey—karyotypes I, II, III, IV, V (*Aotus trivirgatus*)	5/18	29/32	9/9	7/9	0/2	2/2	1/2	1/2	0/2
Owl monkey—karyotypes VI, VII (*Aotus trivirgatus*)	4M/7	5/8	6M/6	3/4					
Squirrel monkey (*Saimiri sciureus*)	1/2	22/22	2/2	M1/2	0/2	2/2	1/1	2/2	0/2
Spider monkey (*Ateles geoffroyi*)	5/12	8/12							
Cebus monkey (*Cebus albifrons*)	0/16	16/16	1/1	1/1	0/1	1/1	1/1	0/1	0/1
Marmoset (*Callithrix jacchus*)	0/3		1/1						
Cotton-top marmoset (*Saguinus oedipus*)	6/6	6/6							
Tamarin (*Saguinus fuscicollis*)	6/6	6/6							
Ring-tailed lemus (*Lemur catta*)	4/11	0/14	11/11	6/11					
Brown lemur (*Lemur fulvus*)	11/11	1/11	5/11	7/11					
Black lemur (*Lemur macaco*)		2/3							
Mongoose lemur (*Lemur mongoz*)		M3/3							
Greater galago (*Galago crassicaudatus*)		M3/3							
Melanotic galago (*Galago argentatus*)		0/3							
Potto (*Perodicticus potto*)		M3/3							
Slow loris (*Nycticebus coucang*)	1/2	3/3	2/2	2/2					
Tree shrew (*Tupaia glis* and *Tupaia belangeri*)		3/6	4/4	M1/4					
Cow (*Bos taurus*)	12/12	5/11	11/12	12/12	0/11	0/11	0/11	6/12	0/11

Table 2. Continued

Buffalo (*Bison bison*)	1/1	0/1	0/1	0/1	0/1				
Goat (*Capra hircus*)	M2/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2	0/2
Donkey (*Equus hemionus*)	1/1	0/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1
Hyrax (*Procavia syriacus*)	0/1	1/1	1/1	0/1	0/1	1/1	0/1	0/1	0/1
Dog (*Canis familiaris*)	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Mouse (*Mus musculus*)‡				0/8	0/8	0/8	0/8		
Chicken (*Gallus domesticus*)	0/1	0/1	0/2	0/2					
Toad (*Bufo marinas*)	0/4	0/4	0/4	0/7	0/4				
Axolotl (*Ambystoma mexicanum*)	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Results are shown as the number of positive reactions over the number of individual tested.
* Orangutang cells tested were from a primary fibroblast line established by skin explant (gift of M. Bobrow).
† The letter M followed by a Roman numeral indicates that number of marginal antibody cross-reactions.
‡ One mouse of each of the *r*, *p*, *d*, *s*, *f*, *q*, *b*, and *k* haplotypes was tested.

reacted with tree shrews and gave positive reactions with two marginal reactions with four out of eight species of prosimians tested, BB7.7 bound to slow loris cells but to no other prosimian cells. Among nonprimate species the number of strong cross-reactions was small but a very significant reaction of W6/32, PA2.6, and MB40.5 as well as of various broadly polymorphic antibodies was seen with cows (Brodsky et al. 1981).

Highly specific antibodies. In general, highly specific polymorphic HLA antibodies did not bind to lymphocytes of nonhuman species as shown in Table 4. The only cross-reactivity shown by any of these antibodies was a few low reactions with individual chimpanzees, gorillas, and gibbons. Fifteen individual cross-reactions were seen with the seven highly polymorphic antibodies out of a total of 526 tested (3 percent) while 368 individual cross-reactions were seen out of 766 tested for the broadly polymorphic antibodies (48 percent). Thus, the two groups of polymorphic HLA antibodies have dramatically different patterns of cross-reactivity with cells of nonhuman species. Broadly polymorphic antibodies cross-react a lot, whereas highly specific polymorphic antibodies show very little cross-reaction.

Discussion

Monoclonal HLA-specific antibodies displayed three different patterns of cross-reactivity with lymphocytes of nonhuman species. These divisions correlated with the patterns of antibody reactions on human cells.

Antibodies which recognize monomorphic determinants of HLA antigens showed extensive monomorphic cross-reactivity with primate cells and scattered monomorphic cross-reactivity in other species, antibodies reacting with broadly

Table 3. Binding of monomorphic HLA-A-, -B-, -C-specific antibodies to peripheral blood cells of nonhuman species

Species	Antibody												
	W6/32	MB40.5	PA2.6	BBM.1	PA2.12	BB7.3	BB7.4	MCLB.3	MCLB.4	BB7.5	BB7.7	BB7.8	BB7.9
Gorilla (*Gorilla gorilla*)	3/3	3/3	3/3	3/3	3/3			3/3	3/3	3/3	3/3	3/3	3/3
Chimpanzee (*Pan troglodytes*)	13/13	4/4	4/4	4/4	4/4	2/2		4/4	4/4	4/4	4/4	4/4	3/3
Gibbon (*Hylobates lar* and *Hylobates concolor*)	8/8	7/7	7/7	0/8	0/7			0/7	0/7	7/7	7/7	7/7	7/7
Orangutang (*Pongo pygmaeus*)*	1/1		1/1	0/1		0/1				1/1		1/1	1/1
Baboon (*Papio anubis*)	6/6	1/1	4/4	0/6		0/1		0/1	0/1	0/1	1/1	1/1	1/1
Crab eating macaque (*Macaca fascicularis*)†	7/7	1/1	1/1	0/7				0/1	0/1	0/1	1/1	1/1	0/1
Blue monkey (*Cercopithicus mitis*)‡	5/5		4/4	0/5						0/2	4/4		0/4
Rhesus monkey (*Macaca mulatta*)	18/18	9/9	16/16	0/18	0/14	0/1	0/1	0/1	0/1	0/1	1/1	2/2	0/2
Owl monkey monkey – karyotypes I, II, III, IV, V (*Aotus trivirgatus*)	0/36	0/3	0/4	0/27				0/3	0/3	0/9	10/10	10/10	0/3
Owl monkey – karyotypes VI, VII (*Aotus trivirgatus*)	12/12		3/3	0/17						0/4	4/4	6/6	
Squirrel momkey (*Saimiri sciureus*)	0/23	0/2	0/2	0/23			0/2			0/2	2/2	2/2	0/2
Spider monkey (*Ateles geoffroyi*)	0/12		0/12	0/12									
Cebus monkey (*Cebus albifrons*)‖	M1/17	0/2	0/16	0/16					0/1	0/1	1/1	1	0/1
Marmoset (*Callithrix jacchus*)	M1/1		0/3	0/1						0/3	1/1	1/1	
Cotton-top marmoset (*Saguinus oedipus*)	M6/7		M6/6	0/1									
Tamarin (*Saguinus fuscicollis*)	M6/7		M6/6	0/1									
Mystax tamarin (*Saguinus mystax*)	0/1			0/1									
Ring-tailed lemur (*Lemur catta*)	13/13	0/3	0/3	0/3				0/3	0/3	0/3	0/13	0/3	0/3
Brown lemur (*Lemur fulvus*)	0/11	0/3	0/3	0/3				0/3	0/3	0/3	0/11	0/3	0/3
Black lemur (*Lemur macaco*)	0/3	0/3	0/3	0/3				0/3	0/3	0/3	0/3	0/3	0/3
Mongoose lemur (*Lemur mongoz*)	M3/3	M3/3	0/3	0/3				0/3	0/3	0/3	0/3	0/3	0/3
Greater galago (*Galago crassicaudatus*)	M3/0	0/3	0/3	0/3				0/3	0/3	0/3	0/3	0/3	0/3
Melanotic galago (*Galago argentatus*)	3/3	M3/3	0/3	0/3				0/3	0/3	0/3	0/3	0/3	0/3
Potto (*Perodicticus potto*)	M3/3	M3/3	0/3	0/3				0/3	0/3	0/3	M3/3	M3/3	0/3
Slow loris (*Nycticebus coucang*)	M3/3	0/1	0/1	0/1				0/1	0/1	0/1	3/3	1/1	0/1
Tree shrew (*Tupaia glis* and *Tupaia belangeri*)	6/6	2/2	M6/6	0/4	0/2					0/1	0/4	0/3	0/3
Cow (*Bos taurus*)	12/12	12/12	12/12	0/12	0/12			0/11	0/11	0/10	M11/11	M11/11	0/11
Buffalo (*Bison bison*)	1/1	1/1	M1/1	0/1	0/1			0/1			0/1	0/1	
Goat (*Capra hircus*)§	0/5	0/2	0/2	0/5	0/2			0/2	0/2	0/2	0/2	0/2	0/2
Donkey (*Equus hemionus*)¶	0/2	0/1	0/1	0/2	0/1			0/1	0/1	0/1	0/1	0/1	0/1

Table 3. Continued

Hyrax (*Procavia syriacus*)	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Dog (*Canus familiaris*)△	0/3	0/1	0/1	0/3	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Rabbit; guinea pig (*Oryctolagus cuniculus; Cavia porcellus*)	0/2			0/2								
Mouse (*Mus musculus*)▽	0/6	0/8		0/6		0/8	0/8					
Chicken (*Gallus domesticus*)	0/2	0/1	0/1	0/2	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
Toad (*Bufo marinas*)○	0/8	0/7	0/7	0/8	0/3	0/3	0/3		0/4	0/4	0/4	
Axolotl (*Ambystoma mexicanum*)	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Results are shown as the number of positive reactions over the number of individuals tested.

* Orangutang cells tested were from a primary fibroblast line established by skin explant (gift of M. Bobrow).

† W6/32 and BBM.1 were also tested against one Moorish macaque (*M. maurus*), one pig-tailed macaque (*M. nemestrina*) one Stumptailed macaque (*M. arctoides*), one Formosan macaque (*M. cyclopis*), and one bonnet macaque (*M. radiata*). Results are tabulated with those for the crab-eating macaque.

‡ W6/32 and BBM.1 were also tested against one African green monkey (*Cercopithicus aethiops*).

‖ The letter M followed by a Roman numeral indicates that number of marginal antibody cross-reactions.

§ W6/32 and BBM.1 were also tested against one pig (*Sus scrofa*) and one sheep (*Ovis aries*). Results are tabulated with those for the goat.

¶ W6/32 and BBM.1 were also tested against one horse (*Equus caballus*). Results are tabulated with those for the donkey.

△ W6/32 and BBM.1 were also tested against one cat (*Felis catus*). Results are tabulated with those for the dog.

▽ W6/32 and BBM.1 were tested against one mouse of each of the *b, k, s,* and *a* haplotypes and two of the *d* haplotype. MB40.5, MCLB.3, and MCLB.4 were tested against one mouse of each of the *r, p, d, s, f, q, b,* and *k* haplotypes.

○ W6/32 and BBM.1 were also tested against one *Xenopus laevis*. Results are tabulated with those for the toad.

polymorphic determinants gave polymorphic cross-reactions with a species distribution similar to the monomorphic antibodies, and antibodies against specific polymorphic determinants showed very little cross-reactivity with nonhuman cells including primate cells. For all three groups of antibodies more cross-reactions were seen with lymphocytes of species evolutionarily close to humans. Within this overall trend, individual determinants appeared to be evolving at different rates, presumably due to different selective pressure and/or molecular constraints. For the monomorphic antibodies, the cross-reactivity seemed to depend on the extent to which the recognized determinant directly involved the β_2-m subunit of HLA antigens. Thus, antibodies reacting with free β_2-m gave limited cross-reaction, those partially inhibited by β_2-m but requiring heavy chain for full antigenicity showed more cross-reactivity and the greatest degree of cross-reactivity was seen with heavy chain-specific antibodies which are not inhibited by β_2m but are nevertheless indirectly dependent on β_2-m binding for the integrity of their antigenic sites. Because β_2-m is a conserved molecule (78 percent homology between human and mouse) it might be expected that those sites on histocompatibility heavy chains with which it interacts might be similarly conserved and that antigenic sites formed as a result of this interaction would be relatively conserved between species (Gates et al. 1981). By contrast an antigenic site on β_2-m unaffected by heavy chain association, might be a site which can tolerate greater residue change without affecting overall molecular structure and would be less conserved, as shown here.

Polymorphic HLA-specific monoclonal antibodies are readily divided into two groups with regard to their reactions with human cells. Highly specific polymorphic antibodies react with only one or two HLA-gene products and therefore with a relatively small number of individuals within the population. Broadly polymorphic

Table 4. Binding of specific polymorphic HLA-A-, -B-, -C-specific antibodies to peripheral blood cells of nonhuman species

Species	Antibody						
	PA2.1	BB7.1	BB7.2	MA2.1	MB40.2	MB40.3	A28M[1]
Gorilla (*Gorilla gorilla*)	0/5	0/6	0/6	1/5	0/4	0/4	0/3
Chimpanzee (*Pan traglodytes*)	0/9	0/15	0/13	0/9	3/9	2/9	6/9
Gibbon (*Hylobates lar* and *Hylobates concolor*)	1/8	0/7	1/7	0/7	0/7	1/7	
Orangutang (*Pongo pygmaeus*)*	0/1	0/1	0/1				
Baboon (*Papio anubis*)	0/5	0/2	0/2		0/1		
Crab- eating macaque (*Macaca fascilurais*)†	0/7	0/1	0/1		0/1		
Blue monkey (*Cercopithicus mitis*)‡	0/1	0/4					
Rhesus monkey (*Macaca mulatta*)‖	0/16	0/16	0/1		0/11	M1/10	
Owl monkey—karyotypes I, II, III, IV, V, (*Aotus trivirgatus*)	0/11	0/7	0/2		0/2		
Owl monkey—karyotypes VI, VII (*Aotis trivirgatus*)	0/9	0/8					
Squirrel monkey (*Saimiri sciureus*)	0/23	0/22	0/1		0/2		
Spider monkey (*Ateles geoffroyi*)	0/12						
Cebus monkey (*Cebus albifrons*)	0/17	0/17	0/1		M1/1		
Marmoset (*Callithrix jacchus*)	0/4						
Cotton-top marmoset (*Sanguinus oedipus*)	0/7						
Tamarin (*Sanguinus fusicolis*)	0/7						
Mystax tamarin (*Sanguinus mystax*)	0/1						
Tree shrew (*Tupaia glis* and *Tupaia belangeri*)	0/2						
Cow (*Bos taurus*)	0/11	0/11	0/11	0/11	0/11		
Goat (*Capra hircus*)	0/2	0/2	0/2	0/2	0/2		
Donkey (*Equus hemionus*)	0/1	0/1	0/1	0/1	0/1		
Hyrax (*Procavia syriacus*)	0/1	0/1	0/1	0/1	0/1		
Dog (*Canus familiaris*)	0/1	0/1	0/1	0/1	0/1		
Mouse (*Mus musculus*)§	0/14	0/6	0/8	0/8			
Chicken (*Gallus domesticus*)	0/1	0/1			0/1		
Toad (*Bufo marinas*)	0/4	0/4			0/7		
Axolotl (*Ambystoma mexicanum*)	0/1	0/1	0/1	0/1	0/1		

Results are shown as the number of positive reactions over the number of individuals tested.

* Orangutang cells tested were from a primary fibroblast lines established by skin explant (gift of M. Bobrow).

† PA2.1 was also tested against one Moorish macaque (*M. maurus*), one pig-tailed macaque (*M. nemestrina*), one stump-tailed macaque (*M. arctoides*), one Formosan macaque (*M. cyclopis*), and one bonnet macaque (*M. radiata*). Results are tabulated with those for the crab-eating macaque.

‡ PA2.1 was tested against one African green monkey (*Cercopithicus aethiops*) and the results are tabulated with those for the blue monkey.

‖ The letter M followed by a Roman numeral indicates that number of marginal antibody cross-reactions.

§ PA2.1 and BB7.1 were tested against one mouse of each of the *b*, *k*, *s*, and *a* haplotypes and two of the *d* haplotype. PA2.1, MA2.1, and MB40.2 were tested against one mouse of each of the *r*, *p*, *s*, *d*, *f*, *q*, *b*, and *k* haplotypes in a separate experiment.

antibodies react with many different HLA-gene products and thus often with the majority of individuals within the population. In this study it has been shown that broadly polymorphic antibodies show considerable cross-reactivity with other species, whereas highly specific polymorphic antibodies do not. Similar results have been obtained with polyclonal antibodies against private and public H-2 antigens (Ivanyi et al. 1976). This is to be expected when one considers how polymorphic antigenic determinants would be generated using a conventional genetic model for the *HLA-A*, *-B*, *-C* loci. Consider a situation in which all the class I HLA-gene products in a population have the identical amino acid at a given position in the amino-acid sequence. A mutation at that position in one gene product in one individual will create two new polymorphic antigenic determinants: (1) a highly specific polymorphic determinant, restricted to a single gene product and a direct of the mutational change, and (2) a broadly polymorphic determinant found on all other gene products, which is structurally not a result of the mutational change. This second antigenic structure represents part of the parent or primordial class I antigen which has been revealed as polymorphic and possibly alloantigenic by the mutation. Thus, as pointed out by Ivanyi (1979) each permissable mutation will produce one public and one private antigen. The private or highly specific structures are the result of recent evolution, the public or broadly polymorphic determinants are older parts of the molecule. Different mutations at the same residue position may create a number of highly specific polymorphic determinants related in this way to a single broadly polymorphic determinant. This broadly polymorphic determinant will be retained in the evolving population unless selection causes one or a combination of the gene products defined by the new highly specific determinants to be fixed. In general this will be unlikely or the tremendous polymorphism observed for class I antigens would not exist. Therefore, one would predict that public or broadly polymorphic antigenic determinants whose origins predate the evolution of the species would exist in current mammalian populations. In consequence, antibodies with specificity for these determinants will frequently react polymorphically and sometimes monomorphically with individuals from other species. On the other hand, antibodies against highly specific polymorphic determinants should only rarely cross-react with other species and in those instances the cross-reactivity may be the result of convergent evolution.

In theoretical papers, Bodmer (1972, 1973) proposed that each individual contained structural genes for all the HLA-A, -B, -C products, arranged as a tandem series within the *HLA* region. The observed polymorphism would then be the result of selective expressions of these genes, which would be inherited in a Mendelian fashion. This model stimulated many investigators to search for situations where this control mechanism was malfunctioning. Hence, the generally abortive quest for alien histocompatibility antigens on cancer cells (Parmiani et al. 1979, Pellegrino et al. 1976).

The experimental basis for the model was that HLA-A, -B, -C polymorphisms were conserved between evolutionarily distant species. This suggested that polymorphisms had arisen before divergence of the species and were selectively maintained in both evolutionary lines. The model avoids the necessity for invoking strong selective forces on polymorphic differences. For in a tandem array of

duplicated genes each HLA-A, -B, -C antigen is a product of a different gene and what are perceived as allotypic differences are in fact akin to the isotypic differences found throughout evolution for the heavy and light chains of immunoglobulin. In interpreting the serological data no distinction was made between the two fundamentally different classes of polymorphic structures we have described above; (1) those created by mutation and (2) those revealed by mutation. However, the implication of Bodmer's arguments was that highly specific antigenic determinants, i. e., those created by mutation, were conserved between such distant species as cattle and humans. For these determinants, e. g., A3 or B7, to be conserved over long periods during evolution would require some special explanation, but if the conserved polymorphic determinants were, as shown here, of the other class and if they represented structures of the ancestral gene products which remained in some allelic products and not in others, then no special explanation would be necessary. The results would merely imply that the mutation rate of class 1 antigens was such that during the period since divergence neither species had accumulated new mutations in all alleles for all potentially variable positions in the amino-acid sequence.

The available amino-acid sequences indicate that certain residue positions can only tolerate limited polymorphism, which results in one of two or three possible amino acids being found in such positions for all gene products of different species (Orr et al. 1979). With a sufficiently high rate of mutation at these positions the same alternative broadly polymorphic antigens would be produced convergently in different species and even within a species. We have previously discussed how this might explain the apparently peculiar properties of the Bw4 and Bw6 antigens (Parham and McLean 1980).

Thus, our results show that the polymorphic structures which are conserved between species are the same structures that define broad polymorphisms within a species. There appears to be no selective conservation of highly specific antigenic determinants and thus no compelling reason for involving complex models to explain *HLA* serology. All available information can be explained by the traditional model of multiple alleles coded for by a small number of loci, i.e., *A*, *B*, and *C*. These results also suggest that reconsideration of the raison d'être of alien antigens might be in order.

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