EVIDENCE FOR BALANCING SELECTION AT HLA

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ABSTRACT

HLA data from the *A* and *B* loci for 22 populations were compared with the neutrality expectations from Ewens' sampling theory. In 25 of 44 cases, there was significantly less homozygosity than expected. Although a number of factors can affect homozygosity in this manner, upon close examination only symmetrical balancing selection appears to be consistent with these data.

THE neutrality theory assumes that different alleles at a locus have equivalent effects on fitness and that the equilibrium heterozygosity is a function of genetic drift and mutation rate. As a result, this model provides theoretical predictions against which observed genetic variation in a population may be compared. A number of statistical tests have been suggested to determine whether genetic variation is consistent with the expectations of the neutrality theory (for a review see EWENS 1977). It appears that some of the data obtained using electrophoresis is consistent with a neutrality model (*e.g.*, WATTERSON 1978a). On the other hand, inversion variation in *Drosophila pseudoobscura* does not appear to be consistent with neutrality (HAYMER and HARTL 1981).

The human histocompatibility system (HLA) was initially examined in detail because of the necessity to match donors and recipients in tissue transplantation. This complex is composed of a large number of genes and is located on chromosome 6. The most well-defined loci are HLA-*A* and HLA-*B*, two genes that determine antigenic factors residing on the surface of many cell types. These loci are 0.8 map units apart, and each is highly variable with large numbers of alleles in nearly every sample that has been examined (see THOMSON 1981 for a review).

Excellent data giving the frequency of genetic variants at these loci in different human populations are available (TERASAKI 1980). In this paper we will compare these observed levels of genetic variation with that expected from the neutrality model. In a large proportion of the samples examined here, the observed homozygosity is significantly less than that expected from neutrality. By examining how various factors affect genetic variation, we are then able to suggest the factors that may be primarily responsible for the extent of variation at these loci.

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TECHNIQUE

EWENS (1972) developed a sampling theory to predict the distribution of alleles observed in a sample of size $2n$ given neutrality. WATTERSON (1978a,b) extended this approach and developed a test that allows the comparison of the observed homozygosity in a sample of size $2n$ genes containing k alleles to the homozygosity expected in a sample drawn from a population in equilibrium under neutrality. (Note that this test does not examine deviations from Hardy-Weinberg proportions but examines the Hardy-Weinberg homozygosity for distributions of allelic frequencies.) In addition, the expected homozygosity and several confidence levels for combinations of $2n$ and k up to 500 and 30, respectively, have been given by ANDERSON (1979). From these tables, if the exact combinations of $2n$ and k observed in the HLA data were not present, the approximate values were linearly interpolated. In cases in which $2n$ was greater than 500, values for 500 were used. This was a conservative value when the alternative hypothesis is that the homozygosity is lower than expected, because with increasing sample size (and a given k), the expected homozygosity increases. For locus B where k was greater than 30 (in two samples there were 31 alleles), confidence values were extrapolated to the higher number of alleles.

DATA

The data utilized were from the Eighth International Workshop on Histocompatibility Testing 1980 (TERASAKI 1980). We have calculated the homozygosity for those "ethnic groups" in which the number of individuals in the sample was greater than 50 ($2n > 100$). In calculating the homozygosity, we have assumed Hardy-Weinberg proportions. It appears that HLA genotypes are near Hardy-Weinberg proportions except for several reports that the homozygosity of haplotypes is lower than the expectation in some populations (e.g., DEGOS *et al.* 1974; BLACK and SALZANO 1981). The unweighted average frequency of unidentified alleles for loci A and B were 0.043 and 0.062, respectively. Data from other HLA loci were not utilized because the proportion of unidentified alleles was quite high in some cases.

RESULTS

TERASAKI (1980) reports data from 22 ethnic groups in which $2n > 100$. Nineteen of these groups are Caucasian, two are African or of African ancestry, and one is Japanese. Tables 1 and 2 give these observed data, the expected homozygosity from neutrality and the significance level for HLA-A and HLA-B, respectively. The observed homozygosity for the A locus ranges from 0.088 to 0.217 and for the B locus from 0.059 to 0.117. In all cases, the observed homozygosity is less than the expected homozygosity assuming neutrality for a given number of alleles and sample size. In several cases, the observed homozygosity is fairly close to the minimum possible ($1/k$) for a given number of alleles, k. For example, the observed homozygosity at the B locus for the Dutch population is 0.059, and the minimum possible is 0.037. The closest to neutrality expectations at the A locus is the Japanese sample. Interestingly, the

TABLE 1

Number of alleles, sample size, expected and observed homozygosity, and the significance level for different populations at the HLA-A locus

Population	k	2n	Homozygosity[a]		P
			Expected	Observed	
Caucasian					
Mexican	16	170	0.190	0.130	
American	18	1734	0.215	0.134	<0.1
Australian	16	318	0.221	0.132	<0.1
Austrian	15	112	0.182	0.132	
Canadian	16	200	0.199	0.149	
Czeck	16	278	0.214	0.115	<0.05
Dutch	16	192	0.196	0.130	<0.1
English	15	576	0.258	0.141	<0.05
French	17	874	0.233	0.139	<0.05
German	17	630	0.233	0.133	<0.1
Hungarian	13	210	0.249	0.203	
Italian	17	1044	0.233	0.118	<0.025
Ashkenazi Jew	16	258	0.211	0.109	<0.01
Non-Ashkenazi Jew	17	124	0.162	0.088	<0.01
Swedish	16	560	0.240	0.132	<0.05
Spanish	17	444	0.222	0.120	<0.05
Swiss	14	174	0.222	0.143	<0.1
Yugoslavian	14	152	0.213	0.180	
Finnish	16	338	0.223	0.133	<0.05
African blacks	17	286	0.204	0.100	<0.01
American blacks	18	372	0.202	0.092	<0.01
Japanese	17	1878	0.233	0.217	

[a] Hardy-Weinberg homozygosity in a sample drawn from a population at equilibrium under neutrality (expected) and the Hardy-Weinberg homozygosity found in different populations (observed).

B locus homozygosity in the Japanese sample is significantly below ($P < 0.025$) the expected level. The closest to neutrality expectations at the *B* locus is the Mexican sample. In this case, the *A* locus is also not significantly different from neutrality.

To determine whether there was any association between the homozygosity values for the *A* and *B* loci, the Pearson product-moment correlation of the difference between expected and observed homozygosity standardized by the expected homozygosity was calculated. The correlation was 0.278 which with 21 degrees of freedom is not significant at the 0.05 level.

Table 3 gives the unweighted mean values for two other measures of genetic variation for the two loci and comparable theoretical values with neutrality (see DISCUSSION). First, notice that the average number of alleles is lower for the *A* locus than for the *B* locus. Second, the two other measures of genetic variation, the number of alleles represented only once in the sample and the frequency of the most common allele, support the evenness of the distribution suggested by the low homozygosity values. In other words, the number of singletons and the frequency of the most common allele are lower than expected from neutrality.

TABLE 2

Number of alleles, sample size, expected and observed homozygosity, and the significance level for different populations at the HLA-B locus

Population	k	$2n$	Homozygosity[a]		P
			Expected	Observed	
Caucasian					
Mexican	22	168	0.133	0.117	
American	31	1734	0.121	0.065	<0.01
Australian	29	318	0.116	0.072	<0.05
Austrian	24	112	0.103	0.075	<0.1
Canadian	25	200	0.122	0.080	<0.1
Czeck	27	280	0.122	0.071	<0.025
Dutch	27	192	0.110	0.059	<0.01
English	28	576	0.136	0.079	<0.05
French	31	874	0.121	0.068	<0.01
German	31	608	0.121	0.066	<0.01
Hungarian	23	210	0.135	0.095	
Italian	29	1044	0.131	0.073	<0.025
Ashkenazi Jew	30	254	0.104	0.075	
Non-Ashkenazi Jew	24	124	0.107	0.067	<0.01
Swedish	31	528	0.121	0.067	<0.01
Spanish	28	444	0.131	0.075	<0.025
Swiss	27	174	0.106	0.074	<0.1
Yugoslavian	26	152	0.106	0.072	<0.1
Finnish	30	338	0.114	0.064	<0.01
African blacks	25	286	0.135	0.089	
American blacks	28	368	0.126	0.077	<0.05
Japanese	29	1900	0.130	0.075	<0.025

[a] See Table 1.

TABLE 3

Unweighted mean values for two other measures of genetic variation and the neutrality expectation for similar k values

	k	No. of singletons	Frequency of most common allele
HLA-*A*	15.4	0.91	0.258
Neutrality	15	3.1[a]	0.4[b]
HLA-*B*	26.7	3.14	0.142
Neutrality	25	5.9[a]	0.234[c]

[a] EWENS (1973).
[b] WATTERSON and GUESS (1977).
[c] MORGAN and STROBECK (1979).

DISCUSSION

In the samples examined here, the observed homozygosity for both the HLA-*A* and HLA-*B* loci at the HLA system was always less than the expectation from neutrality and was statistically significantly less at the 0.05 level in 25 of 44 cases. The heterozygosity for these loci is often stated as being high, so it is

important to note that in a number of cases it is not significantly different from neutrality. However, the variation observed at these loci appears to be the result of differences at as few as 20 amino acids that determine antigenic specificity (LOPEZ DE CASTRO *et al.*, 1982). Compared with the inversions in *Drosophila pseudoobscura*, which may consist of several hundred genes, the extent of variation is quite high.

Table 4 gives a number of factors that have been suggested as important at HLA loci, categorized by their expected effect on homozygosity relative to neutrality. Not included here are such possibilities as unusual gene conversion events (OHTA 1982; WEISS *et al.* 1983) or more than one active gene at each locus. These factors, if present, may increase the total amount of variation, but it is not clear how they would affect the frequency distribution of the alleles. Several factors may increase the homozygosity, but we will only briefly discuss them here because their influence on the homozygosity at the HLA loci level must be of less importance than factors decreasing homozygosity.

First, genetic hitchhiking was suggested by MAYNARD SMITH and HAIGH (1974) as a mechanism that could increase homozygosity, whereas OHTA and KIMURA (1975) contended that hitchhiking would not result in a large deviation from neutrality. These differing conclusions appear to depend upon the initial conditions in the population (THOMSON 1977). Second, populations may not be at the equilibrium homozygosity, which, for a neutrality model, is determined by genetic drift and mutation. For example, a selectively favorable allele may have increased to a high frequency in the past and consequently reduced the number of alleles (and heterozygosity) at the locus (EWENS and GILLESPIE 1974). Third, directional selection favoring a particular allele, or, for that matter, asymmetrical balancing selection, will increase homozygosity over that expected for neutrality. Fourth, some suggestions have been made that particular HLA haplotypes may cause diseases. Of course, if these were selectively disadvantageous then they would be in low frequency and consequently increase the overall homozygosity as compared with neutrality. However, some such haplotypes are in high frequency suggesting that other factors may be important in determining their frequencies. Finally, intragenic recombination appears to have a small influence on homozygosity in a given sample, but its effect is to slightly increase homozygosity (MORGAN and STROBECK 1979; HUDSON 1983).

The homozygosity may be decreased by several factors. First, the samples may be stratified, that is, have some population structure. (Remember we are not considering deviations from Hardy-Weinberg proportions but the Hardy-Weinberg homozygosity for a distribution of allelic frequencies in a sample.) Using simulation. EWENS and GILLESPIE (1974) have shown that the effect of "geographical subdivision, unless extremely strong," is not substantial. In some samples analyzed here, such as the American and the non-Ashkenazi jew, high stratification would be possible. Although these two samples have low homozygosity, they are not different from a number of other samples, such as the Dutch and the Swedish samples, that *a priori* may be expected to be more homogeneous. In addition, the allelic frequencies for the different Caucasian samples are quite similar (the genetic distances are small and lumping of all

TABLE 4

Factors that may affect the homozygosity at the HLA loci

Increase	Decrease
Hitchhiking	Stratification or migration
Nonequilibrium	Unidentified alleles
Directional selection	Bottleneck
Disease-causing antigens	Balancing selection
Intragenic recombination	Heterozygote advantage
	Frequency dependent

groups only slightly reduces homozygosity) so that stratification within a sample would require more variation among subpopulations within a sample than between samples, an unlikely expectation. Finally, the lack of a significant correlation between the *A* and *B* homozygosity values suggests that the factors affecting the two loci may not be entirely the same. One might expect that stratification would affect both loci in a similar fashion and result in a significant positive correlation between the loci.

Second, the presence of unidentified alleles may lead to a decrease in homozygosity compared with that expected from neutrality (EWENS and GILLESPIE 1974). EWENS and GILLESPIE considered this effect for the KIMURA and OHTA (1973) charge state model, sometimes assumed for electrophoretic data. However, if we limit ourselves to variations in the antigenic region, there is only a small proportion of nonidentification that occurs because some alleles are not yet categorized into known antigenic classes. Presumably, these unidentified alleles do have antigenic capabilities, but appropriate antisera are not yet available. However, if the frequency of unidentified alleles influences homozygosity in a systematic way, then this frequency should be correlated with the measure of homozygosity used previously, that is, the expected minus the observed homozygosity standardized by the expected homozygosity. Such correlation values for the *A* and *B* loci are 0.236 and 0.085, respectively, neither significantly different from zero.

Third, a bottleneck in population size sometime in the past could have led to a relatively greater reduction in the number of alleles than a reduction in heterozygosity (NEI, MARUYAMA and CHAKRABORTY 1975) and does appear important for HLA in South American Indian populations (HEDRICK, 1983). As a result, k would be reduced, making the observed homozygosity lower than the neutrality expectation. If this is the explanation for the observed deviation from neutrality, then bottlenecks must have occurred in a large proportion of populations (or perhaps previous to the formation of different groups). Additionally, the lack of a significant correlation between the *A* and *B* homozygosity values suggests that the factors affecting the two loci may be different. As with stratification, one might expect that a bottleneck would affect both loci in a similar way and result in a positive correlation. Finally, the frequency of the most common allele appears to be lower than the neutrality expectation. For example, an extrapolation from the calculations of WATTERSON and GUESS (1977) indicates that for $k = 15$ and $2n = 300$ the frequency of the most common

allele should be about 0.4, much higher than the value of 0.258 observed for HLA-*A* (see Table 3). In addition, the simulations of MORGAN and STROBECK (1979) give the frequency of the most common allele for $k = 25$ and $2n = 250$ under neutrality at 0.234, much higher than the value of 0.142 observed for HLA-*B*. If anything, one might expect a bottleneck to increase the frequency of the most common allele (R. CHAKRABORTY, personal communication), an expectation that appears to be inconsistent with these data.

Fourth, some type of balancing selection may reduce the observed homozygosity. Such selection should be fairly symmetrical, e.g., all homozygotes having lower and nearly equal fitness values and all heterozygotes having higher and nearly equal fitness values. If the fitness values do not fit such a pattern, then the distribution of alleles at equilibrium may result in a homozygosity near or even below neutrality. One example of a symmetrical constant fitness model is one in which all heterozygous haplotypes have a higher fitness than all homozygous haplotypes (BLACK and SALZANO 1981). BLACK and SALZANO suggest that such an advantage may result because "disease resistance by specific antigens seems to require the presentation to the responding cells of one of their own histocompatibility antigens as a part of the total antigenic pattern that the responding cell recognizes (BLANDEN 1980). Responding cells that carry two antigens for each locus would have more opportunities to respond than would homozygous cells." A frequency-dependent selection model in which genotypes with a rare allele have an advantage would work in much the same way. BODMER (1972) suggested that a new allele would allow greater protection against pathogens than more common alleles to which pathogens may have evolved resistance.

As a result of this consideration of possible causes for the observed reduced homozygosity it appears that some form of symmetrical balancing selection, and not stratification, nonidentity of alleles, or past bottlenecks is the explanation most consistent with the genetic variation at the HLA loci *A* and *B* in these populations.

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