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HLA class I and class II allele distribution in the Peruvian population

Key words:
Amerindians; HLA; Peruvian; Quechua

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Abstract: The distribution of HLA-A, -B, -C, -DRB1 and -DQB1 alleles in the Peruvian population was studied and compared with those of other populations in order to provide further information about their anthropological origin. Our data are consistent with the Mestizo character of this population. In terms of genetic distance Peruvians are closest to Bolivians, which is in agreement with the geographical location and the cultural and anthropological background of the two human groups. Several HLA-B alleles originally described in genetically isolated Amerindian tribes are also present in the sample studied here. This fact and the reported finding of these alleles in several Amerindian groups suggests that they were present in the first wave of humans that populated South America (Paleoindians) before they split to give rise to the different South American tribes.

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Amerindian populations are characterized by the restricted polymorphism of the HLA class I and class II allelic series (1–3). Mechanisms as bottleneck effect, endogamy or epidemics have been evoked to explain this fact. The limited HLA polymorphism of these population groups has been considered a factor contributing to their susceptibility to diseases introduced by Europeans (4). However, the total number of alleles in a population is not a direct indication of the ability to respond against pathogens and, thus, it is not necessarily a disadvantage (5, 6).

The presence of new HLA-B alleles in Amerindians has been considered to be a characteristic of populations from South America, Central America and southwestern North America (5). To explain the high frequency of these new alleles in spite of the aforementioned limited HLA polymorphism, a process called allele turnover has been evoked by Parham et al. (5). According to these authors, new alleles tend to supplant older ones rather than supplement them. This fact led some authors to enunciate the hypothesis of a more rapid evolution of HLA-B in isolated populations, specifi-

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cally in South America (7–9). As more Amerindian populations are studied with the appropriate DNA-based technologies the geographical range of new alleles will emerge, permitting this hypothesis to be tested.

Considering that the differential distribution of HLA alleles in different populations is a fact supported by numerous reports, and with the aim of characterising the Peruvian population better, we have analysed the distribution of both HLA class I and class II genes in this human group. This study will contribute to gain knowledge on the relationship of the different South American populations and on the history of migrations that populated the New World.

Material and methods

Population studied and DNA class I and class II typing

We have studied a sample of 148 unrelated subjects taking part in a house-to-house clinic-epidemiological survey carried out in rural settlements, in the villages of Tiabaya and Caraveli, located in the Southern Peruvian province of Arequipa, an area with a high rate of infection by *Trypanosoma cruzi*. All the individuals were Mestizo of Quechua and Hispanic origin.

Genomic DNA was obtained from peripheral blood leukocytes by a modified salting-out method (10). HLA-A, -B and -C loci were typed by polymerase chain reaction using sequence-specific primers (PCR-SSP) performed as described (11) using primer mixes provided by the 12th IHW Anthropology component (HLA-A, -B) and AHS#9 (HLA-C) (12, 13), following the Workshop protocols (13). Some individuals were additionally typed for HLA-A, -B and/or -C with the corresponding PCR-SSP kit manufactured by Dynal (Dynal, Oslo, Norway) which in a few cases resolves at allelic level. HLA-B*35 and B*40 alleles were typed by sequence-based typing (SBT), a high-resolution technique, as described by Pozzi et al. (14). The analysis of sequences and the HLA-B allele assignment was performed using a Perkin Elmer-Applied Biosystems MathTools™ software (Perkin Elmer, Foster City, CA, USA) for HLA class I SBT.

HLA-DRB1 was typed by PCR-restriction fragment length polymorphism (RFLP) using amplification-created restriction sites (ACRS) (15). Because of the informativeness as population markers of DRB1*04 subtypes, we subtyped for DRB1*04 alleles, also by PCR-RFLP/ACRS (15). DQB1 allele distribution was determined by reverse PCR using sequence-specific oligonucleotides (SSO) with the kit manufactured by Dynal (Dynal RELI™; Dynal) and following the manufacturers' instructions.

Statistical analysis

Phenotypic frequencies are expressed as percentage of individuals bearing a given specificity and gene frequencies were derived from them (16). Heterozygosity of the loci studied was calculated from the DNA results as described elsewhere (17). Linkage disequilibrium of two-locus haplotypes was determined according to Baur and Danilovs (16), χ^2 was calculated using a 2×2 contingency table and the level of significance was corrected for the number of alleles studied in the two allelic series, according to the formula $p_c = 1-(1-p)^n$, where n is the number of alleles studied in the first locus multiplied by the number of alleles studied in the second locus. Genetic distances were calculated according to Nei (18). Frequency of haplotypes of two, three and five loci were obtained by direct counting and expressed as indicated in the corresponding tables. Linkage disequilibrium of five-locus haplotypes was calculated as described elsewhere (19). The degree of genetic admixture has been calculated as the cumulated frequency of the non-typically Amerindian alleles for the HLA-A and -B loci (20). This value corresponds to the sum of the frequencies of those alleles for every locus (20). Frequency of alleles in each allelic family was expressed as a percentage of the whole number of individuals in that family; frequency of haplotypes was obtained by direct counting and expressed as indicated in the corresponding tables.

Results

HLA class I and class II allele frequencies

The frequency distribution of HLA-A, -B, -C, -DRB1 and -DQB1 alleles is shown in Table 1. The analysis of the frequencies demonstrates that the population is under Hardy-Weinberg equilibrium. The most frequent class I alleles of the sample studied were A*02, B*35 and Cw*04, all of them showing a gene frequency higher than 30%. During the PCR-SSP typing of HLA-C, a peculiar pattern was found in individual Peru15. The characterization of the allele responsible for this pattern led to the description of a new HLA-C allele, namely Cw*1508, which seems to be associated with HLA-B*51 in the individual from whom it was cloned (21).

The class II alleles more frequently observed were DRB1*08, *04, *09, and *14, representing 68.1% of the sample. The high frequency of DRB1*08 (18.8%) and DRB1*14 (13.81%) is a constant finding in practically all isolated Amerindian populations (22). DRB1*09 is less extended in the indigenous populations of South America. However, we found a frequency (17.57%) similar to those in Kolla (12%) and Chiriguanos (17.9%) from Northern Argentina

HLA class I and class II phenotypic and allele frequencies in a sample of 148 individuals from the province of Arequipa in Southern Perú. Only represented alleles are indicated

HLA-A allele	P.F.	G.F.	HLA-B allele	P.F.	G.F.
A*01	8.1	4.05	B*07	4.73	2.37
A*02	79.1	54.45	B*08	4.73	2.46
A*03	7.4	3.98	B*13	1.35	0.68
A*11	8.8	4.39	B*14	3.38	1.69
A*23	4.1	2.03	B*15	20.27	10.56
A*24	18.9	10.38	B*18	1.35	0.68
A*26	2.0	1.01	B*22	0.68	0.34
A*29	5.4	2.70	B*27	1.35	0.68
A*30	3.4	1.69	B*35	57.43	34.46
A*31	7.4	3.98	B*37	0.68	0.34
A*32	4.0	2.03	B*38	2.70	1.35
A*36	0.7	0.34	B*39	8.78	4.69
A*66	1.4	0.67	B*40	15.54	10.57
A*68	13.5	6.76	B*42	0.68	0.34
A*69	0.7	0.34	B*44	12.16	6.08
A*80	1.4	0.67	B*45	2.03	1.01
ABL		0.53	B*46	0.68	0.34
Heterozygosity		67.95	B*47	1.35	0.68
			B*48	14.87	8.21
HLA-C allele	P.G.	G.F.	B*49	1.35	0.68
Cw*01	24.32	13.25	B*50	1.35	0.68
Cw*02	2.70	1.35	B*51	14.87	8.21
Cw*03	1.35	0.67	B*52	1.35	0.68
*0303	2.03	1.01	B*53	1.35	0.68
*0304	13.51	7.09	B*55	1.35	0.68
Cw*04	60.13	37.42	B*57	0.68	0.34
Cw*05	6.76	3.38	B*58	0.68	0.34
Cw*06	4.73	2.36	BBL		0.18
Cw*07	20.94	11.09	Heterozygosity		83.73
Cw*08	15.54	7.94			
Cw*12	4.05	2.03	HLA-DQB1	P.F.	G.F.
Cw*15	12.16	6.73	*02	15.06	8.42
Cw*16	4.05	2.03	*0301	41.09	22.16
CBL		3.65	*0302	32.19	17.69
Heterozygosity		81.14	*03032	30.82	16.68
			*0402	38.35	20.14
HLA-DRB1 allele	P.F.	G.F.	*0501	4.11	2.05
*01	2.74	1.37	*0502	0.68	0.34
*02	9.58	5.08	*0503	4.11	2.05
*03	10.27	5.43	*06011	1.37	0.68
*04	32.87	17.92	*0602	4.11	2.05
*07	8.90	4.58	*0603	9.58	5.34
*08	35.61	18.80	*0604	1.37	0.68
*09	32.19	17.57	*0609	2.05	1.02
*10	1.37	0.68	DQBL		0.64
*11	9.59	4.93	Heterozygosity		83.98
*13	12.32	6.32			
*14	25.34	13.81			
DRBL		3.45			
Heterozygosity		86.83			

P.F. and G.F.: Phenotypic and allele frequencies, respectively

Table 1

(23), and in the Cayapa Indians (20%) from Ecuador (24). Outside South America this specificity only achieves high frequencies in populations of Asia-Oceania (25–27).

The DR4 specificity is coded for by more than 20 different sequences or subtypes that show marked differences in their interpopulational distribution. In Peruvians, the DRB1*04 allele frequencies are 38% for DRB1*0404, 24% for DRB1*0407, 12% for DRB1*0403, 8% for DRB1*0405, 6% for DRB1*0408 and 4% for DRB1*0401, *0410 and *0411. Alleles DRB1*0403, *0404, *0407, and *0411 were previously found in isolated Amerindians and all of them are represented in Peruvians, making up almost 80% of DRB1*04 in the sample.

Given the high frequency in Peruvians of B*35 and B*40, 82 of the 85 individuals typed as B*35 and 22 of the 23 individuals typed as B*40 were characterized by SBT (Table 2). Considering that B*15 was present in 21 and B*48 in 13 of the individuals typed by SBT, the frequency of alleles of these two groups was also determined. Among the remaining HLA-B alleles found during the course of the SBT typing but not quantified, HLA-B*3903 (7) and *5104 (8) had been described in genetically isolated Amerindian tribes.

Haplotype frequencies

HLA-A-B-C-DRB1-DQB1 haplotypes are shown in Table 3. A*02-B*35-Cw*04-DRB1*08-DQB1*0402 was the most frequent haplo-

Frequency of alleles B*35, B*40, B*15 and B*48 allelic families

HLA-B*35 allele	Frequency	HLA-B*40 allele	Frequency
*3501	40.2%	*40011	9%
*3502	7.3%	*4002	36.4%
*3503	1.2%	*4004ˢ	18.1%
*3504ˢ	9.8%	*4008ˢ	50%
*3505ˢ	40.2%		
*3506ˢ	2.4%	HLA-B*15 allele	Frequency
*3508	3.7%	*1501	42.8%
*35091ˢ	12.2%	*1504ˢ	28.6%
*3510ˢ	2.4%	*1507	4.8%
*3511ˢ	1.2%	*1508	4.8%
*3512ˢ	1.2%	*1522ˢ	14.3%
*3521ˢ	1.2%	*1539	4.8%
HLA-B*48 allele	Frequency		
*4801	69.2%		
*4802ˢ	15.4%		
*4803ˢ	15.4%		

Frequency is expressed as a percentage of the whole number of individuals studied in every allelic family. ˢ: Alleles originally described in Amerindians

Table 2

HLA-A-B-C-DRB1-DQB1 haplotypes in a sample of 148 Peruvian individuals

Haplotype					H.F.	L.D.
A*02	B*35	Cw*04	DRB1*08	DQB1*0402	9.6	9.3
A*02	B*35	Cw*04	DRB1*09	DQB1*03032	3.0	2.8
A*02	B*15	Cw*01	DRB1*14	DQB1*0301	2.5	2.4
A*02	B*15	Cw*01	DRB1*09	DQB1*03032	2.5	2.4
A*02	B*35	Cw*04	DRB1*07	DQB1*02	1.9	1.8
A*24	B*15	Cw*01	DRB1*14	DQB1*0301	1.7	1.6
A*01	B*08	Cw*07	DRB1*03	DQB1*02	1.7	1.6
A*02	B*35	Cw*04	DRB1*04	DQB1*0302	1.7	1.4

H.F.: Haplotype frequency expressed as a percentage. L.D.: Linkage disequilibrium (calculated as indicated in reference 19)

Table 3

type in Peruvians, already described as being present in South American Amerindians, Mexican Indians and Mexican Mestizo population (30). This haplotype and A*02-B*35-Cw*04-DRB1*09-DQB1*03032, which probably corresponds to A*0211-B*3505-Cw*0401-DR9-DQ3 described by Parham et al. in the Guarani population (5), can be considered typical Amerindian haplotypes. A*01-

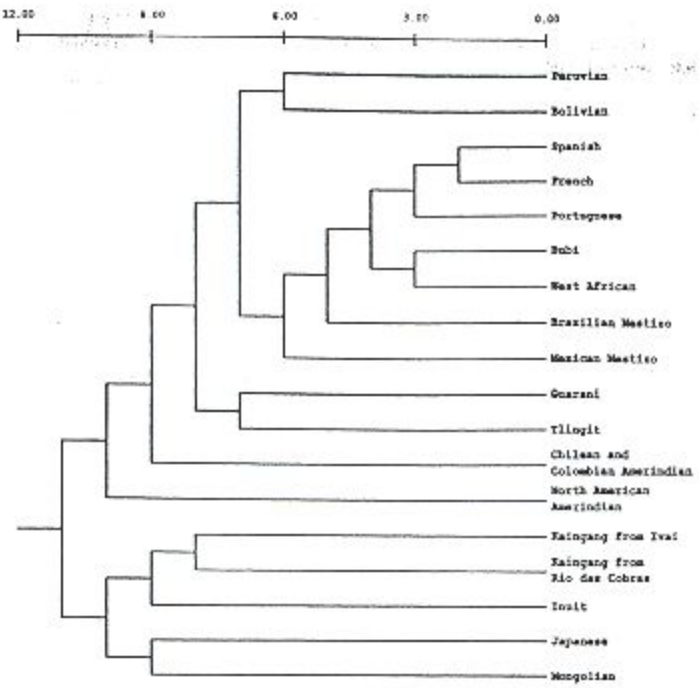

Fig. 1. Dendrogram constructed according to the neighbour-joining method method using the genetic distance data obtained with HLA-A and -B gene frequencies (Table 4). The length of the branches does not match real genetic distance.

Genetic distances ($\times 10^3$) among several Amerindian, Asian, African, Caucasoid and Mestizo populations calculated with the HLA-A and B gene frequencies

	PRV	BLV	GRN	KIV	KRC	CH/C	NAM	TLG	INU	SPA	POR	FRN	BUB	WAF	MXM	BZM	JAP	MON
PRV	000																	
BLV	039	000																
GRN	202	212	000															
KIV	879	811	1053	000														
KRC	660	624	846	017	000													
CH/C	139	159	379	940	735	000												
NAM	269	215	477	749	581	157	000											
TLG	275	251	215	1297	1051	198	159	000										
INU	658	668	659	813	749	480	469	271	000									
SPA	254	315	542	1326	1006	387	495	465	606	000								
POR	206	244	514	1029	802	285	425	429	473	034	000							
FRN	301	352	549	1190	939	343	488	406	407	041	038	000						
BUB	692	639	904	2131	1843	930	1099	914	1221	439	493	534	000					
WAF	411	317	622	1319	1133	531	529	510	838	370	322	388	289	000				
MXM	156	162	311	893	709	136	280	229	416	123	083	096	478	301	000			
BZM	160	150	397	957	753	239	294	293	485	049	041	063	440	231	062	000		
JAP	426	428	639	1069	891	225	269	233	290	448	368	394	1049	673	268	340	000	
MON	292	334	515	1062	839	236	278	219	231	227	183	170	724	401	166	171	126	000

HLA-A and -B gene frequencies were used to calculate the genetic distances, as indicated in Material and methods. PRV: Peruvians, BLV: Bolivian Amerindians, GRN: Guarani, KIV: Kaingang from Ivai, KRC: Kaingang from Rio das Cobras, CH/C: Chilean and Colombian Amerindians, NAM: North American Indians, TLG: Tlingit, INU: Inuit, SPA: Spaniards, POR: Portuguese, FRN: French, BUB: Bubi, WAF: West Africans, MXM: Mexican Mestizo, BZM: Brazilian Mestizo, JAP: Japanese, MON: Mongolians

Table 4

B*08-Cw*07-DRB1*03-DQB1*02 constitutes a typical Caucasian haplotype (3) and its presence in the studied population is probably due to gene flow from Caucasians.

Genetic distance

The genetic distance of the Peruvian sample studied and several ethnic groups of Amerindian origin (3, 20) (Guarani, Kaingang from Ivai, Kaingang from Rio das Cobras, Bolivian, Chilean and Colombian, North American Indian, Tlingit, Inuit), Caucasoid (3) (Spanish, French, Portuguese), Mongoloid (3) (Japanese, Mongolian), Negroid (3, 29) (Bubi, West African) and Mestizo populations (3) (Mexican and Brazilian) was determined considering HLA-A, -B and -DR frequencies.

In terms of genetic distance, when it is obtained considering HLA class I frequencies (Table 4) the Peruvian group studied is closest to Bolivian Amerindians, followed by Chilean and Colombian Amerindians, and the Mestizo populations of Mexico and Brazil. The corresponding dendrogram constructed according to the neighbour-joining method (Fig. 1), shows that the populations compared are organized in two main groups. In one of them Peruvians are close to the native population from Bolivia, and both are grouped with Caucasoids, Negroids, some Amerindian native populations and the Amerindian Mestizo populations studied. The two Kaingang groups, Inuit, Japanese and Mongolian are clustered in the second group.

When the genetic distance is calculated with DR frequency data (Table 5), Peruvians are close to Japanese and again close to Bolivian Amerindians, followed by the Mexican Mestizo population. The populations are distributed in two main groups in the corresponding dendrogram obtained by the neighbour-joining method (Fig. 2). All the American native populations are clustered in the first group with the only exception of Tlingit, who are grouped with Caucasian, Negroid and Amerindian Mestizo populations.

Discussion

The distribution of HLA alleles in the Peruvian sample studied is consistent with its anthropological origin, that is, they present an HLA frequency distribution with a typical Amerindian profile (9, 20, 24, 32). However, they show more variability in every allelic

Genetic distances ($\times 10^3$) among several Amerindian, Asian, African, Caucasoid and Mestizo populations calculated with the HLA-DR gene frequencies

	PRV	BLV	GRN	KIV	KRC	CH/C	NAM	TLG	INU	SPA	POR	FRN	BUB	WAF	MXM	BZM	JAP	MON
PRV	000																	
BLV	182	000																
GRN	545	220	000															
KIV	395	146	401	000														
KRC	380	165	356	013	000													
CH/C	325	166	387	571	627	000												
NAM	929	820	178	1272	1010	1161	000											
TLG	392	726	1271	1668	1537	1167	991	000										
INU	287	152	426	642	708	091	998	598	000									
SPA	429	508	586	1017	947	427	720	792	382	000								
POR	400	638	818	1148	1023	826	741	451	526	059	000							
FRN	332	496	512	988	853	629	465	467	423	050	030	000						
BUB	420	889	1362	1720	1405	1443	876	094	760	481	208	236	000					
WAF	399	631	816	823	644	797	825	795	649	254	198	171	323	000				
MXM	250	250	356	572	532	421	513	489	219	096	091	064	348	230	000			
BZM	365	504	569	966	819	540	589	625	395	052	054	031	298	087	078	000		
JAP	148	151	161	392	355	250	366	623	271	436	510	349	659	492	238	363	000	
MON	325	400	322	669	588	472	402	919	439	115	171	101	583	243	108	096	205	000

HLA-DR gene frequencies were used to calculate the genetic distances, as indicated in Material and methods. PRV: Peruvians, BLV: Bolivian Amerindians, GRN: Guarani, KIV: Kaingang from Ivai, KRC: Kaingang from Rio das Cobras, CH/C: Chilean and Colombian Amerindians, NAM: North American Indians, TLG: Tlingit, INU: Inuit, SPA: Spaniards, POR: Portuguese, FRN: French, BUB: Bubi, WAF: West Africans, MXM: Mexican Mestizo BZM: Brazilian Mestizo, JAP: Japanese, MON: Mongolians

Table 5

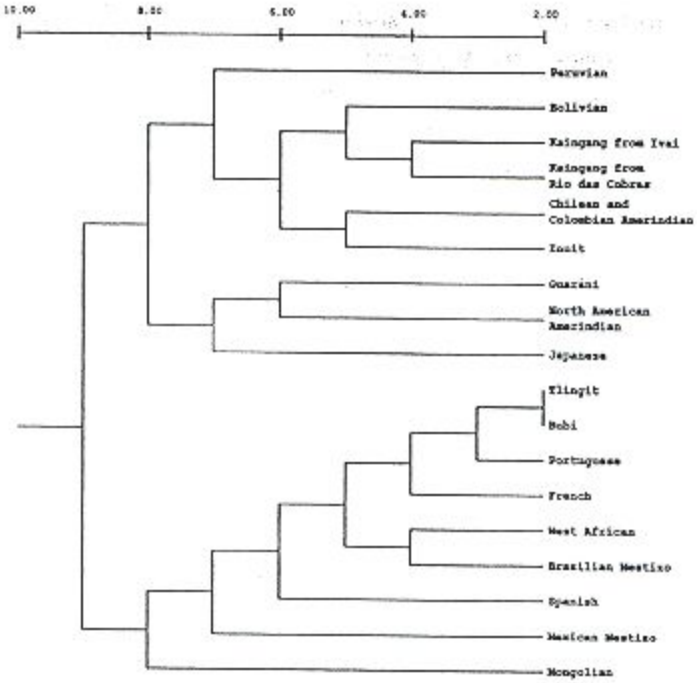

Fig. 2. **Dendrogram constructed according to the neighbour-joining method using the genetic distance data obtained with HLA-DRB1 gene frequencies (Table 5). The length of the branches does not match real genetic distance.**

series than expected in South American Indian populations, a finding that is in agreement with their constituting a group of Quechua and Hispanic admixture.

The first study of the HLA antigen distribution of Quechua Indians from Peru was carried out during the course of the International Histocompatibility Workshop held in 1972 (33). The HLA distribution described then also showed an Amerindian profile, the blank for HLA-A being less than 1% and the blank for HLA-B being 11%, a fact that indicates that the former allelic series was better defined than the second one. Although the scarcity of alleles found in the above mentioned Quechua sample is coincident with the small number of alleles described in Amerindians (1, 24, 32), the presence of A1 and A11 (G.F. of 0.01 in both cases) points to a certain degree of admixture with Caucasians (5, 9).

The HLA-A allele distribution, well defined in Quechua, as previously mentioned, is very similar to the corresponding locus in the Peruvian sample under study. However, the cumulated frequency of HLA-A non-typically Amerindian specificities (9, 20) in Quechua is 9%, being 23.93% % in our sample. This indicates a lower degree of genetic admixture in Quechua than in the Peruvian sample described here, a finding that is consistent with the anthropological background of both populations.

Allele B*4802 typed serologically as B*35 and was proposed to be a chimaera between B*3501 and B*4801 (7). The leader and α1 domain of *4802 correspond to B*4801, while α2, α3, transmembrane and cytoplasmic domains are identical to B*3501 (7). This allele may have resulted of a single recombination event between B*4801 and B*3501 at some point of intron 2. Since HLA-C is located 3′ to HLA-B and in the same transcriptional orientation, the resulting allele would be expected to be associated with Cw04. In fact, Cw*04 was linked with B*4802 in the two individuals bearing this allele in Peruvians and in the Waorani individual in which it was described (7).

Parham et al. described the haplotype B*1504-Cw*0303 in Guarani, and B*1520-Cw*0304 in Kaingang (5). They probably correspond respectively to haplotypes B62-Cw9 and B62-Cw10 found in Mongoloid and Caucasoid populations (3). In our hands only one case out of the 21 B*15 individuals SBT typed, showed this allele associated with Cw*0304 (B*1501-Cw*0304). In the remaining 20 individuals B*15 was associated with Cw*01. This association was found in Mongoloid Asian populations, North American Indians (Navajo Indians) and Brazilian Caucasians (3). The presence of haplotype B62-Cw1 in the last population could be due to gene flow from neighbouring Amerindian populations.

The new allelic variants described in genetically isolated South American tribes but not found in North American Amerindians, or in African, Oriental or Caucasian populations, led Parham and Watkins (7, 8) to suggest that the diversification of human MHC class I loci may take place more rapidly than previously estimated. In fact, they suggested that the HLA-B allelic variants they described would have been generated after the arrival of Paleoindians in South America. Alleles B*1504, *4004, *3504, *3505, *3506, *35091, *3903, *4802 and *5104 were among the new HLA-B alleles found in isolated South American Amerindians that were evoked to advance this hypothesis. However, the presence of all of them in the population under study points to a wider distribution than expected when they were reported in highly endogamous tribes.

Satz (34) and Ogawa (35) questioned the above mentioned hypothesis as a result of the finding of some of these alleles in Asian (3) and Caucasian populations (36). This fact and the wide distribution they present in South America, where many of them can be found in tribes living in geographically distant areas (30, and this report), point to the existence of these alleles in the population groups that migrated out of Asia.

The proposal of a rapid evolution of HLA-B in South America is based on results obtained by high-resolution typing techniques. Considering the lack of HLA data obtained in Caucasian, Asian and African populations with this level of resolution, and the presumably low frequency of the above mentioned alleles, a definite conclusion cannot be made until the appropriate DNA typing technology is used to study these populations.

With respect to HLA class II, there are some alleles belonging to

the DR2, DR4, DR6, DR8 and DR9 groups that are considered to be typical of Amerindians (30), although different populations may present different subsets of this groups of alleles. In this respect, the high frequency of DRB1*04, DRB1*08, DRB1*09 and DRB1*14 in Peruvians confirms the anthropological characterization of this group as typically Amerindian. The same conclusion is reached when DQB1 typing of Peruvians is compared with the data of the 12th IHW (30), again the most frequent alleles in Amerindian populations are also found in Peruvians: DQB1*0301, *0302, *03032 and *0402. These DQB1 alleles were seen in all the Amerindian samples of population analyzed during the 12th IHW, with the exception of DQB1*03032, that was frequent in Kolla, an indigenous group from Argentina (30). The Mestizo character of our group is confirmed by the presence of typical Caucasian haplotypes such as A*01-B*08-Cw*07-DRB1*03-DQB1*02 and A*29-B*44-Cw*16-DRB1*07-DQB1*02.

In terms of genetic distance, Peruvians are closest to Bolivians, a fact that results in the two populations being grouped together in the dendrogram shown in Fig. 1. This fact is in agreement with the geographical location and the cultural and anthropological background of the two human groups. The close relationship between Peruvians and several Amerindian groups shown in Fig. 2 is coincident with the anthropological background of these populations, the only exception being Inuit, an Aleut-speaking people who are grouped with Chilean and Colombian Amerindians, and Tlingits that belong to the Na-Dene liguistic group are clustered with Bubi. The Peruvians studied being a Mestizo population, their clustering with American native groups points to a small degree of admixture with Hispanics, a possibility that is coincident with a cumulated frequency of non-typically Amerindian alleles of 23.93% for HLA-A and 23.12% for HLA-B.

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