ANALYSIS OF HUMAN LEUKOCYTE ANTIGENS OF CLASS II-DR IN BRAZILIAN CHILDREN AND ADOLESCENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

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OBJECTIVE: To analyze the frequency of human leukocyte antigens class II-DR in children and adolescents with systemic lupus erythematosus.

PATIENTS AND METHODS: Fifty-five Brazilian systemic lupus erythematosus children and adolescents and 308 healthy individuals were studied. Gender, race, and age of onset of systemic lupus erythematosus were recorded. The human leukocyte antigens typing of class II-DR was carried out by polymerase chain reaction amplification with sequence-specific primers (PCR-SSP). Data were analyzed statistically using the chi square test with Yates' correction, Fisher's exact test, and Bonferroni's correction.

RESULTS: Human leukocyte antigen-DR 15 was the most frequently detected antigen in this group of children and adolescents, and it also occurred more frequently in the female group, in children with onset of systemic lupus erythematosus between 0 and 9 years and between 10 to 14 years, and in the Black race group, but these associations were not statistically significants.

CONCLUSION: In this group of children and adolescents with a high degree of racial admixture, we could not verify a significant association between human leukocyte antigens class II-DR and systemic lupus erythematosus.

DESCRIPTORS: **Systemic lupus erythematosus. Human leukocyte antigens. Children and adolescents.**

INTRODUCTION

A complex genetic and immunologic network participates in the pathogenesis of systemic lupus erythematosus (SLE)[1,2]. Among the genetic factors, the human leukocyte antigens (HLA) have earned special attention in the literature, and a series of works have reported an increased frequency of the HLA-DR2 (15/16), DR3 (17/18), DR7, and DR9 in patients with SLE[3-7].

The relationships established to date between the HLA and SLE were in adult populations or in mixed populations of adults and children, with few works in literature involving only children.

The objective of this study was to analyze the frequency of the HLA class II-DR in Brazilian children and adolescents with SLE.

PATIENTS AND METHODS

A total of 55 children and adolescents with a diagnosis of SLE[8] were studied and followed up. Patients were included in the study after informed and written consent and following approval of the study by the ethics commission. The patients' demographic characteristics are presented in table 1.

The control group comprised 308 healthy and unrelated individuals; it was established using the DNA bank from the Laboratory of Immunology of the Heart Institute, Hospital das Clínicas, Faculty of Medicine, University of São Paulo.

The typing of HLA class II-DR was carried out by polymerase chain reaction amplification with sequence-spe-

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cific primers (PCR-SSP). The method was based on the procedure developed by Olerup and Zetterquist[9].

Statistical analysis included the chi square test with Yate's correction of continuity and Fisher's exact test. Confidence limits of 95% ($P = 0.05$) were used. The significant results were multiplied by the total number of tested antigens (14 antigens), thereby obtaining a corrected value of P, termed Pc (Bonferroni's correction)[10-12].

RESULTS

In this group of 55 Brazilian children and adolescents with SLE, the HLA-DR15 was detected most frequently; it was present in 18 patients (33%). The second most frequently detected antigen was HLA-DR7, present in 14 patients (26%), and the third was HLA-DR13, present in 13 patients (24%).

The comparisons between the frequencies of HLA-DR in the group of patients with SLE and in the control group are presented in table 2.

It was not possible to establish an association between HLA and the group of children and adolescents with SLE under study, in spite of the suggestion of an association with HLA-DR15 ($P = 0.0186$), which was not confirmed after Bonferroni's correction (Pc = 0.260).

In the 15 patients with age at disease onset between 0 and 9 years, HLA-DR13 and HLA-DR15 were detected most frequently ($P = 0.309$ and $P = 1.0$, respectively) and were present in 33% of the patients. In the 35 patients aged between 10 and 14 years, HLA-DR15 was the most frequently detected ($P = 0.978$) and was present in 34% of the patients. In the 5 patients with ages between 15 and 19 years, HLA-DR13 ($P = 0.581$), HLA-DR4 ($P = 0.258$), and HLA-DR7 ($P = 0.592$) were the most frequently detected antigens.

Table 1 - Demographic characteristics of the 55 children and adolescents with SLE.

Demographic characteristics			
Sex (n)	Female		46
	Male		09
Age at disease onset (years) Mean / range			10.4 / 1 to 17
Age at disease onset Stratified (n)	0 to 9 years (n=15)	Female	14
		Male	01
	10 to 14 years (n=35)	Female	29
		Male	06
	15 to 19 years (n=05)	Female	03
		Male	02
Age at inclusion to study (years) Mean / range			14 / 8 to 20
Follow up (years) Mean / range			3.5 / 1 to 13
Race – Caucasian (n = 25)	Female		22
	Male		03
Black (n = 07)	Female		05
	Male		02
Asian (n = 01)	Female		01
	Male		00
Mixed (n = 22)	Female		18
	Male		04

Table 2 - Frequency of HLA-DR in the 55 children and adolescents with SLE and in the 308 individuals comprising the control group.

HLA-DR	Patients (n = 55)	Controls (n = 308)*	P	Pc
DR1	18%	22.2%	0.602	-
DR15	**33%**	**18%**	**0.0186**	**0.260**
DR16	4%	6.3%	0.550	-
DR17	15%	18.4%	0.606	-
DR18	0%	3.3%	0.370	-
DR4	20%	21.8%	0.867	-
DR11	13%	23.4%	0.112	-
DR12	5%	1.3%	0.0737	-
DR13	**24%**	**27.7%**	**0.621**	**-**
DR14	5%	7.5%	0.779	-
DR7	**26%**	**21.8%**	**0.706**	**-**
DR8	16%	10%	0.254	-
DR9	5%	5.4%	0.763	-
DR10	5%	2.9%	0.402	-
Blank	11%	5.2%	0.136	-
DR51	36%	23.8%	0.0696	-
DR52	62%	67.4%	0.532	-
DR53	51%	45.2%	0.518	-

Blank = Non-identified antigens
*Samples supplied by the DNA bank from the Laboratory of Immunology- Heart's Institute, Hospital das Clínicas, Faculty of Medicine, University of São Paulo[13].

The relationships between HLA-DR, gender, and ethnic group are presented in table 3. HLA-DR 15 was the most frequently detected antigen in the female group and in the Black race group, but again, after Bonferroni's correction, there was no association between HLA-DR and gender or ethnic group.

Table 3 - Frequency of HLA-DR in the 55 children and adolescents with SLE, according to gender and ethnic group.

HLA- DR	Number of patients	Female	Male	Caucasian	Black	Asian	Mixed
DR1	10	7	3 (*P*=0.339)	8 (*P*=0.0315 *P*c=0.412)	-	-	2
DR 15	18	17 (*P*=0.240)	1	7	5 (*P*=0.0316 *P*c=0.412)	-	6
DR 16	2	2	-	2	-	-	-
DR 17	8	5	3 (*P*=0.113)	4	1	-	3
DR 18	0	-	-	-	-	-	-
DR 4	11	9	2	8 (*P*=0.0905)	-	-	3
DR 11	7	7	-	2	2	-	3
DR 12	3	3	-	1	-	-	2
DR 13	13	10	3 (*P*=0.427)	4	3	-	6
DR 14	3	3	-	2	-	-	1
DR 7	14	11	3 (*P*=0.678)	4	2	-	8 (*P*=0.229)
DR 8	9	7	2	3	1	1	4
DR 9	3	3	-	1	-	-	2
DR 10	3	3	-	2	-	-	1
Blank	6	5	1	2	-	1	3
DR 51	20	19	1	9	5	-	6
DR 52	34	28	6	13	6	-	15
DR 53	28	23	5	13	2	-	13
Total	55	46	9	25	7	1	22

Blank = Non-identified antigens

DISCUSSION

One of the main problems in analyzing the studies regarding HLA is related to the methodology adopted. The characterization of the HLA can be accomplished using the serologic method (for detecting DR2) or PCR amplification with sequence-specific primers (for detecting DR15 and DR16) or using the specific sequences of oligopeptides (for detecting DRB1*1501 to 1506 and DRB1*1601 to 1608). These techniques have different sensitivities, which hinders an effective comparison between the studies.

The Brazilian population, and especially those who reside in the city of São Paulo, is characterized by a high degree of racial admixture. The ethnic origin of these individuals is very difficult to establish. The majority possesses different percentages of Caucasian, Black, and Amerindian ancestry. Therefore, it is necessary to determine which HLA are associated with SLE in a population characterized by this degree of miscegenation.

Despite exhaustive studies in patients with SLE, the sensitivity and specificity of the associations between HLA and SLE vary in the diverse studies and different ethnic groups, rendering the results inconclusive[14,15]. Analysis of various studies suggests a strong association between the HLA-B8/DR3/DQ2/C4A0 and B7/DR2/DQ1 haplotypes and SLE[3,15-24]. HLA-DR2 (15/16) and HLA-DR3 (17/18), taken individually, are also associated with SLE, but the relative risk conferred by these is low (from 2 to 3)[16].

SLE is rare in childhood, yet the risk of SLE in the children of patients with SLE is about 10%, demonstrating a genetic component of the disease.

In infants, SLE develops with clinical manifestations that maintain some differences when compared to those observed in adults[1,2]. These differences in manifestation raise some questions regarding the possibility that SLE in childhood is a disease with its individual genetic characteristics and may be different from SLE in adults.

Lehman et al.[25] studied 71 children with SLE and observed that the onset of the disease before 10 years of age was strongly related to the presence of antibodies to Ro/SSA in the maternal blood, suggesting that the antibody to Ro/SSA can influence the immunological development of the fetus.

In this group of children with SLE, we observed that the distribution of the various HLA-DR was similar to the control group, except for HLA-DR15 which was present in 33% of the children with SLE and in 18% of the control group; however, this relationship was not statistically significant after Bonferroni's correction (P = 0.0186 and Pc = 0.260). HLA-DR7 and DR13 were the second and the third most frequently detected antigens, respectively, but again, these correlations were not statistically significant.

In Brazil, Silva et al.[26] studying 93 patients with SLE with ages between 13 and 66 years, observed a higher frequency of HLA-DR3 (17/18) (P = 0.003), a result that differs from that observed in our study and from that reported by Fernandes et al.[27] in a study

with 56 adult SLE patients, in which HLA-DR2 (15/16) was the most frequently detected antigen (P <0.005).

The association of HLA-DR7 and HLA-DR13 with SLE was observed in a few studies, such as that by Wilson et al.[7] in African Americans and that by Cowland et al.[28] in Denmark.

Regarding the age of SLE onset, some authors describe an increased frequency of the HLA-B8/DR3/DQ2/C4A0 haplotype in patients among which the onset of the disease occurred after 35 years of age. Other authors have described an increased frequency of the HLA-B7/DR2/DQ1 haplotype in patients whose SLE appeared before 25 years of age and that presented serious nephritis[3]. However, Barron et al.[29] in a study of children with SLE did not corroborate these findings.

Likewise, we did not observe any relationship between the age at onset of SLE and HLA. However, all of our patients presented onset of the SLE before 25 years of age, and HLA-DR2 (15/16) was present in 37%, therefore demonstrating a behavior similar to that described above.

It is interesting to underscore that in this study, HLA-DR15 was the most frequently detected antigen in the 0 to 9-year-old and 10 to 14-year-old age groups, but this was not the case among the adolescents from 15 to 18 years of age.

The associations between the HLA and SLE vary between the different ethnic groups. HLA-DR3 is associated with SLE in Caucasians, and HLA-DR2 is associated with Black, Chinese, and Japanese populations[6,15]. In Caucasian children with SLE, an association was observed with HLA-DR3(DRB1*0301), and in Blacks, there was an association with HLA-DR2(DRB1*1503/DRB1*1501) / DRB5*0101 / DQA1*0102 / DQB1*0602[29].

In our study, HLA-DR1 and HLA-DR4 were the most frequently detected antigens in the Caucasian patients. There is no report of an association between HLA-DR1 and SLE in the literature, either among Caucasians, Black, or Asians[6], while HLA-DR4 is described as being associated with SLE only in Indians[30]. According to the literature, 75% of Caucasian patients with SLE present the HLA-DR2 and/or HLA-DR3 antigens. We observed that 52% of our Caucasian patients presented these antigens.

HLA-DR15 was the most frequently antigen in the Black race patients of this study. The HLA-DR2 (15/16) has been associated with Black race SLE patients both in Black race people from America as from South Africa[24, 31, 32].

Among the Asian patients, an association has been described between SLE and HLA-DR9 in Chinese patients from Singapore [5] and in Koreans[20] and with HLA-DR15 in Japanese[19], Chinese[23], and Korean patients[20]. Our only Asian patient presented HLA-DR8 in homozygosis. There is no association described between SLE and HLA-DR8.

In the patients of the mixed-race group, HLA-DR7 was the most frequently occurring antigen. There are descriptions of an association between HLA-DR7 and SLE only in Black race people from America[6,7].

It is interesting to observe that in our study, there was a prevalence of HLA-DR7 in the mixed-race group and of HLA-DR15 in the Black race group, suggesting that perhaps the group considered to be mixed race here may in fact not be characterized by a predominant miscegenation of Black race with other races.

In the present study, we observed associations between HLA and SLE ethnic groups that were different from those observed in the literature. This difference is probably due to the fact, as already stated, that racial admixture in Brazil is very prominent, which hinders a comparison of our ethnic groups with those of other countries. (Table 4)

SLE occurs more frequently among women, mainly after puberty, with a female:male ratio of 8:1. The higher incidence of SLE in women has been associated with hormonal factors, such as estrogen, which could influence the onset of this pathology. In children, female prevalence also occurs; perhaps genetic factors, especially concerning HLA, could have some influence on the higher incidence of SLE in the female sex. The ratio between the female and male sex among the children and adolescents with SLE in this study was 5.1:1, a finding similar to the ratio reported in the

Table 4 - Relationship between the HLA and SLE racial groups.

Author	Ethnic Groups			
	Caucasian	Black	Asian	Mixed
Arnett[3]	HLA-DR2/DR3	-	-	-
Fernades et al.[27]	HLA-DR2	-	-	-
Reivelle et al.[32]	-	HLA-DR2	-	-
Rudwaleit et al.[31]	-	HLA-DR2	-	-
Barron et al. [29]	HLA-DR3	HLA-DR2	-	-
Rudwalet et al. [5] and Hong et al.[20]	-	-	HLA-DR9	-
Hashimoto et al.[19] and Doherty et al.[23]	-	-	HLA-DR15 (2)	-
Wilson et al.[7]	-	HLA-DR7	-	-
Liphaus (present study)	HLA-DR1/DR4	HLA-DR15 (2)	HLA-DR8	HLA-DR7

literature, and there was a uniform distribution of HLA-DR in both the female and male groups. (Table 3)

The majority of studies do not report a relationship between the HLA and gender; however, Doherty et al.[23] observed that the HLA-DR15 antigen was detected in all the men in their study population.

In view of the discrepancies observed in the various studies referred to in the literature, it is possible that HLA have little or no influence on the mechanisms of etiopathology of SLE. While possible, this fact seems improbable because of the close relationship between HLA and the immunological system. Perhaps in the near future, methods with greater sensitivity and more specific probes will establish clearer correlations than those seen to date.

RESUMO

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LIPHAUS B de L e col. - Análise dos antígenos de histocompatibilidade leucocitária de classe II-DR em crianças e adolescentes brasileiros com lúpus eritematoso sistêmico. **Rev. Hosp.Clín. Fac. Med. S. Paulo 57**(6):277-282 2002.

OBJETIVO: Analisar a freqüência dos antígenos de histocompatibilidade leucocitária de classe II-DR em crianças e adolescentes com o lúpus eritematoso sistêmico.

PACIENTES E MÉTODOS: Cinqüenta e cinco crianças e adolescentes lúpicos brasileiros e 308 indivíduos sadios foram estudados. Os sexos, os grupos étnicos e as idades de início da doença foram anotados. A tipagem de histocompatibilidade leucocitária de classe II-DR foi realizada pela reação de polimerase em cadeia com amplificação de sondas de seqüência específica (PCR-SSP). Na análise estatística foram utilizados o teste de qui-quadrado com correção de Yates, o teste exato de Fisher e a correção de Bonferroni.

RESULTADOS: A histocompatibilidade leucocitária-DR15 foi a mais freqüente neste grupo de crianças e adolescentes, sendo também mais freqüente nas mulheres, nas crianças com idade de início da doença entre zero e nove anos e entre 10 e 14 anos e nas crianças de raça negra, mas estas correlações não foram estatisticamente significativas.

CONCLUSÃO: Neste grupo de crianças e adolescentes com alto grau de miscigenação não pudemos observar associação significativa entre os antígenos de histocompatibilidade leucocitária de classe II-DR e o lúpus eritematoso sistêmico.

DESCRITORES: **Lúpus eritematoso sistêmico. Antígenos de histocompatibilidade leucocitária. Crianças e adolescentes.**

REFERENCES

1. CASSIDY, J.T. - Systemic lupus erythematosus, juvenile dermatomyositis, scleroderma and vasculitis. In: KELLEY WN; HARRIS ED; RUDY S et al. - (Ed) - **Textbook of Rheumatology.** 5th ed. Philadelphia, Pennsylvania, Saunders, 1997. p1241-5.

2. CASSIDY JT & PETTY RE - Systemic lupus erythematosus. In: **Textbook of Pediatric Rheumatology.** 3rd ed. Philadelphia, Pennsylvania, Saunders, 1995. p 260-322.

3. ARNETT FC - The genetics of human lupus. In: WALLACE DJ & HAM BH ed. - **Dubois Lupus Erythematosus**. 5th ed. Philadelphia, Lea, 1997. p 77-117.

4. HAHN BH - An overview of the pathogenesis of systemic lupus erythematosus. In: WALLACE DJ & HAM BH ed. - **Dubois Lupus Erythematosus**. 5th ed. Philadelphia, Lea, 1997. p 69-75.

5. RUDWALEIT M; GIBSON K; WORDSWORTH P et al. - HLA associations of systemic lupus erythematosus in Chinese from Singapore. **Ann Rheum Dis** 1995; **54**(8): 686-7.

6. SCHUR PH - Genetics of systemic lupus erythematosus. **Lupus** 1995; **4**: 425-37.

7. WILSON WA; SCOPELITIES E; MICHALKI JP - Association of HLA-DR7 with antibody to SSA(Ro) and disease susceptibility in blacks with systemic lupus erythematosus. **J Rheumatol** 1984; **11**: 653-7.

8. TAN EM; COHEN AS; FRILS JF et al. - The 1982 revised criteria for the classification of SLE. **Arthritis Rheum** 1982; **25**: 1271-7.

9. OLERUP O & ZETTERQUIST H - HLA-DR typing by PCR amplification with sequence-specific primers (PCR-SSP) in 2 hours: An alternative to serological DR typing in clinical practice including donor-recipient matching in cadaveric transplantation. **Tissue Antigens** 1992; **39**: 225-35.

10. BENGTSSON BO & THOMSON G - Measuring the strength of associations between HLA antigens and diseases. **Tissue Antigens** 1981; **18**: 356-63.

11. FUKUDA K; SUGAWA K; WAKISAKA A et al. - Statistical detection of HLA and disease association. **Tissue Antigens** 1985; **26**: 81-6.

12. SVEJGAARD A; JERSILD C; STAUB NIELSEN L et al. - HLA antigens and disease statistical and genetic considerations. **Tissue Antigens** 1974; **4**: 95-105.

13. GOLDBERG AC; CHIARELLA JM; MARIN MLC et al. - Molecular typing of HLA class II antigens in a São Paulo population. **Genet Mol Biol** 1998; **21**(3): 301-5.

14. ADAMS D - How the immune system works and why it causes autoimmune diseases. **Immunology Today** 1996; **17**(7): 300-2.

15. ARNETT FC - Genetic aspects of human lupus. **Clin Immunol Immunopathol** 1992; **63**(1): 4-6.

16. HAHN BH - Pathogenesis of systemic lupus erythematosus. In: KELLEY WN; HARRIS ED; RUDY S et al. – (Ed.) - **Textbook of Rheumatology**. 5th ed. Philadelphia, Saunders, 1997. p. 1015-27.

17. DAVIES EJ; HUTCHINGS CJ; HILLARBY MC et al. - HLA-DP does not contribute towards susceptibility to systemic lupus erythematosus. **Ann Rheum Dis** 1994; **53**(3): 188-90.

18. DAVIES EJ; STEERS G; OLLIER WER et al. - Relative contributions of HLA-DQA and complement C4a loci in determining susceptibility to systemic lupus erythematosus. **Br J Rheumatol** 1995; **34**: 221-5.

19. HASHIMOTO H; NISHIMURA Y; DONG RP et al. - HLA antigens in Japanese patients with systemic lupus erythematosus. **Scand J Rheumatol** 1994; **23**(4): 191-6.

20. HONG GH; KIM HY; TAKEUCHI F et al. - Association of complement C4 and HLA-DR alleles with systemic lupus erythematosus in Koreans. **J Rheumatol** 1994; **21**(3): 442-7.

21. BATCHELOR JR - Systemic lupus erythematosus and genes within the HLA region. **Br J Rheumatol** 1993; **32**(1):13-5.

22. GOLDSTEIN R; SENGAR DPS - Comparative studies of the major histocompatibility complex in French Canadian and non-French Canadian Caucasians with systemic lupus erythematosus. **Arthritis Rheum** 1993; **36**(8): 1121-7.

23. DOHERTY DG; IRELAND R; DEMAINE AG et al. - Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus in southern Chinese. **Arthritis Rheum** 1992; **35**(6): 641-6.

24. HARTUNG K; BAUR MP; COLDEWEY R et al. - Major histocompatibility complex haplotypes and complement C4 alleles in systemic lupus erythematosus. Results of a multicenter study. **J Clin Invest** 1992; **90**(4): 1346-51.

25. LEHMAN TJA; REICHLIN M; SANTNER TJ et al. - Maternal antibodies to Ro(SSA) are associated with both early onset of disease and male sex among children with systemic lupus erythematosus. **Arthrtis Rheum** 1989; **32**(11): 1414-20.

26. SILVA LM & DONADI EA - Is immunogenetic susceptibility to neuropsychiatric systemic lupus erythematosus (SLE) different from non-neuropsychiatric SLE ? **Ann Rheum Dis** 1996; **55**: 544-7.

27. FERNANDES SRM; PERSOLI LB; MARQUES SBD et al. - HLA antigens and susceptibility to systemic lupus erythematosus in Brazilian patients. **Lupus** 1995; **4:**43s.

28. COWLAND JB et al. - DNA polymorphism of HLA class II genes in systemic lupus erythematosus. **Tissue Antigens** 1994; **43**: 34-7.

29. BARRON KS; SILVEMAN ED; GONZALES J et al. - Clinical, serologic, and immunogenetic studies in childhood-onset systemic lupus erythematosus. **Arthritis Rheum** 1993; **36**(3): 348-54.

30. MEHRA NK; PANDE I; TANEJA V et al. - Major histocompatibility complex genes and susceptibility to systemic lupus erythematosus in northern India. **Lupus** 1993; **2**(5): 313-4.

31. RUDWALEIT M; TIKLY M; GIBSON K et al. - HLA class II antigens associated with systemic lupus erythematosus in black South Africans. **Ann Rheum Dis** 1995; **54**: 678-80.

32. REVEILLE JD; BARGER BO; HODGE TW - HLA-DR2-DRB1 allele frequencies in DR2-positive black Americans with and without systemic lupus erythematosus. **Tissue Antigens** 1991; **38**(4): 178-80.

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