

LACK OF ASSOCIATION BETWEEN PARACOCCIDIOIDOMYCOSIS AND HLA HISTOCOMPATIBILITY ANTIGENS

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ABSTRACT

A study on association between HLA and disease was carried out. Forty caucasian patients with clinical and laboratorial diagnosis of Paracoccidioidomycosis (PCM) and eighty healthy caucasian individuals, used as controls, were typed for the antigens HLA-A, -B, -Cw, -DR and -DQ.

There was a positive association of antigens HLA-A1 ($p = 0.050$), -A3 ($p = 0.014$), -B8 ($p = 0.014$), -Cw7 ($p = 0.020$), -DQw2 ($p = 0.014$) and -DQw3 ($p = 0.019$) in patients, and a negative association of antigens HLA- Cw3 ($p = 0.032$), -DR1 ($p = 0.019$) and -DQw1 ($p = 0.003$) in the same group (compared to the controls, and without correction for the number of antigens tested (50)). The results suggest a fragile association of these HLA antigens with the disease, since other factors may also influence the genetic susceptibility to PCM. No significant association was demonstrated in this study when the value of P was corrected according to Svejgaard and Ryder (*HLA and Disease*, J. Dausset and A. Svejgaard, eds., 1977).

INTRODUCTION

Paracoccidioidomycosis (PCM) is a chronic granulomatous disease cause by the fungus *Paracoccidioides brasiliensis* (Splendore, 1912).

PCM is most frequently encountered in Brazil, Venezuela, Colombia and Argentina. The endemic centre is in Brazil, mainly in the sub-tropical areas with

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predominant agricultural activity. This disease is most frequent in agricultural workers aged 20-60 years old (Del Negro *et al.*, 1982).

Not all individuals infected with PCM present active disease. This suggests that conditions to establish the disease also depend on host susceptibility. Genetic factors, among others, have demonstrated influence in the development of the disease (Fava Netto *et al.*, 1965).

Considering that the HLA complex contains genes which control not only the main transplant antigens but also various related biological properties, such as genes for immune response to thymus-dependent antigens and genes for genetic susceptibility to various diseases (Munro and Bright, 1976), we decided to study a possible association of HLA antigens with PCM.

MATERIAL AND METHODS

Blood samples were collected from forty caucasian morphologically patients with clinical laboratorial diagnosis of PCM (pulmonary lesions) diagnosed at Hospital de Clínicas in Curitiba and at Hospital Universitário in Londrina, Paraná, Brazil.

The control group consisted of eighty healthy individuals selected according to ethnical characteristics, sex, occupation and geographical origin. Among the eighty individuals 58.75% were of caucasian origin, 8.75% were brazilian white people and 32.50% were not ethnically identified. The whole group has some activity in the rural area, in Northeast Paraná State, with a 1:19 proportion of women:men.

The HLA-A, -B, -Cw, -DR and -DQ classification was carried out according to the microlymphocytotoxicity test (Terasaki and McClelland, 1964), after the isolation of lymphocytes from peripheral blood (Boyum, 1968) and (Werner *et al.*, 1977).

The HLA anti-sera, used in the classification, were obtained from the immunogenetics Laboratory of Maringá State University and from Histocompatibility laboratories, by means of exchange of sera and was also bought from suppliers (Pel Freez).

To detect possible differences between patients and controls for phenotypic frequencies of HLA antigens, classical tests of association study were used (Svejgaard *et al.*, 1974).

For each specificity, a 2x2 contingency table was made and the chi-square test was applied. For values above 3.84, values estimates were made with Fisher's exact test.

RESULTS

The frequency of antigen specificity for *loci* HLA-A, -B, -Cw, -DR and -DQ of controls and patients are listed in Tables I, II, III, IV and V, respectively.

The statistical analysis of uncorrected data demonstrated a significant increase in the frequency of antigens HLA-A1, -A3, -B8, -Cw7, -DQw2 and -DQw3 in PCM patients compared to control individuals, and, a decrease in the frequency of antigens HLA-Cw3, -DR1 and -DQw1.

Table I - HLA-A frequency in patients with PCM and in controls.

HLA	Patients (40)		Controls (80)		Significance		
	n	%	n	%	P	Pc	χ^2
A1	14	35.00	15	18.80	0.050	NS	3.84
A2	15	37.50	37	46.30	NS		0.83
A3	14	35.00	13	16.30	0.014	NS	5.38
A9	10	25.00	26	32.50	NS		0.71
A10	4	10.00	7	8.80	NS		0.05
A11	3	7.50	8	10.00	NS		0.20
A28	3	7.50	6	7.50	NS		0.00
A29	2	5.00	4	5.00	NS		0.00
A30	5	12.50	7	8.80	NS		0.31
A31	4	10.00	4	5.00	NS		1.55
A32	3	7.50	3	3.80	NS		0.79
A33	1	2.50	3	3.80	NS		0.13
A36	0	0.00	1	1.30	NS		0.50

NS - not significant; P - uncorrected value; Pc - value corrected for the number of comparisons (50).

DISCUSSION

There was a prevalence of the male sex over the female sex. Recent studies with PCM suggest that women are less susceptible to the disease, mainly due to the influence of female hormonal factors. Estradiol, for example, inhibits the change of the fungus from its mycelial form to the parasitic yeast form (Loose *et al.*, 1983).

Agricultural workers were most affected by PCM (62.5% of patients), agreeing with the literature (Londero, 1982).

The frequency of HLA antigens was compared between patient and control groups, to determine a possible association between an HLA antigen and PCM.

Table II - HLA-B frequency in patients with PCM and in controls.

HLA	Patients (40)		Controls (80)		Significance		
	n	%	n	%	P	Pc	χ^2
B5	9	2.25	21	26.30	NS		0.31
B7	10	25.00	10	12.50	NS		3.00
B8	12	30.00	10	12.50	0.014	NS	5.45
B12	6	15.00	11	13.80	NS		0.03
B13	3	7.50	3	3.80	NS		0.79
B14	3	7.50	13	16.30	NS		1.77
B15	3	7.50	5	6.30	NS		0.07
B16	1	2.50	2	2.50	NS		0.00
B17	4	10.00	6	7.50	NS		0.02
B18	3	7.50	8	10.00	NS		0.20
B21	2	5.00	5	6.30	NS		0.08
B22	0	0.00	3	3.80	NS		1.54
B27	3	7.50	5	6.30	NS		0.07
B35	8	20.00	19	23.80	NS		0.22
B37	0	0.00	2	2.50	NS		1.02
B40	5	12.50	14	17.50	NS		0.50
B48	0	0.00	1	1.30	NS		0.50

NS - not significant; P - uncorrected value; Pc - value corrected for the number of comparisons (50).

Table III - HLA-Cw in patients with PCM and in controls.

HLA	Patients (33)		Controls (80)		Significance		
	n	%	n	%	P	Pc	χ^2
Cw1	1	3.00	1	1.30	NS		0.43
Cw2	8	24.20	9	11.30	NS		3.09
Cw3	2	6.10	17	21.30	0.032	NS	3.85
Cw4	9	27.30	23	28.80	NS		0.03
Cw5	3	9.10	3	3.80	NS		1.33
Cw6	7	21.20	11	13.80	NS		0.97
Cw7	14	45.40	18	22.50	0.020	NS	4.57
Cw8	1	3.00	7	8.80	NS		1.16

NS - not significant; P - uncorrected value; Pc - value corrected for the number of comparisons (50).

Table IV - HLA-DR frequency in patients with PCM and in controls.

HLA	Patients (40)		Controls (80)		Significance		
	n	%	n	%	P	Pc	χ^2
DR1	2	5.00	16	20.00	0.019	NS	4.71
DR2	11	27.50	16	20.00	NS		0.86
DR3	13	32.50	14	17.50	NS		3.44
DR4	4	10.00	13	16.30	NS		0.86
DR5	15	37.50	21	26.30	NS		1.61
DR6	3	7.50	14	17.50	NS		2.19
DR7	15	37.50	20	25.00	NS		2.02
DR8	0	0.00	2	2.50	NS		1.02
DR10	1	2.50	1	1.30	NS		0.25

NS - not significant; P = uncorrected value; Pc = value corrected for the number of comparisons (50)

Table V - HLA-DQ frequency in patients with PCM and in controls.

HLA	Patients (40)		Controls (80)		Significance		
	n	%	n	%	P	Pc	χ^2
DQw1	15	37.50	52	65.00	0.003	NS	8.18
DQw2	21	52.50	25	31.30	0.014	NS	5.09
DQw3	17	42.50	19	23.80	0.019	NS	4.46

NS - not significant; P = uncorrected value; Pc = value corrected for the number of comparisons (50)

Published works about PCM so far are rare and they present conflicting results. Some authors suggest positive association with the antigen HLA-B12 (González *et al.*, 1983), others with HLA-A9 and -B13 (Restrepo *et al.*, 1983) and yet another group suggests association with HLA-B40 (Lacerda, 1988). Svejgaard and Ryder (1977) suggest that the value of P should be corrected by the number of antigens tested.

Braun (1979), referring to studies about associations of 20 or more antigens, found that they should attain an uncorrected value of $P = 0.0005$, to be significant at a level of 1% when corrected (Pc).

Without correction, the incidence of six antigens was higher in PCM patients and lower for three antigens. The corrected probability did not reach a P value sufficient to establish significance to 5% level, even though they were significant at $\alpha = 0.05$ when analysed singly.

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RESUMO

Foi realizado um estudo de associação HLA e doença, onde 40 pacientes com diagnóstico clínico e laboratorial de Paracoccidioidomicose (PCM) e, 80 indivíduos brancos, clinicamente saudáveis, usados como controles, foram tipados para os antígenos HLA-A, -B, -Cw, -DR e -DQ.

Os resultados obtidos mostraram uma associação positiva dos antígenos HLA-A1 (P = 0.050), -A3 (P = 0.014), -B8 (P = 0.014), -Cw7 (P = 0.020), -DQw2 (P = 0.014) e DQw3 (P = 0.019) nos pacientes e uma associação negativa dos antígenos HLA-Cw3 (P = 0.032), -DR1 (P = 0.019) e -DQw1 (P = 0.003) no mesmo grupo, comparados aos controles e, sem correção pelo número de antígenos testados (50). Os resultados sugerem uma fraca associação destes antígenos HLA com a doença, uma vez que outros fatores podem também estar influenciando na susceptibilidade genética à PCM. Se corrigido o valor de P, segundo Svejgaard e Ryder (*HLA and Disease*, J. Dausset and A. Svejgaard, eds., 1977), nenhuma associação é demonstrada neste estudo.

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