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HLA–Gm/κm interaction in sarcoidosis. Suggestions for a complex genetic structure

M. Martinetti*, J-M. Dugoujon**, C. Tinelli***, A. Cipriani+, A. Cortelazzo++, L. Salvaneschi*, L. Casali#, G. Semenzato‡, M. Cuccia++, M. Luisetti#

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ABSTRACT: The aetiology of sarcoidosis is still unknown. Environmental exposures are believed to interact with genetic factors in determining the pattern of sarcoidosis presentation, progression and prognosis.

The frequency of serological polymorphism of immunoglobulin G heavy chain (Gm) and κ light chain (κm) markers in 107 patients with biopsy-proven sarcoidosis and in 227 controls, and their interactions with histocompatibility leukocyte antigen (HLA) class I, II, and III markers, were studied.

A "protective" effect of the Gm(3 5*) phenotype in the sarcoid group *versus* controls (p-value for number of specificities tested (p_c)=0.05, odds ratio 0.15) and a reduced frequency of Gm(3 23 5*) in patients with advanced chest radiographic stage (Chi-squared (two degrees of freedom)($\chi^2_{(2df)}$ 17.61, pc=0.0058) were observed. With reference to epistatic interactions, the combination Gm(3 23 5*)/BfS had a "protective" effect towards stage II ($\chi^2_{(2df)}$ 13.86, p_c=0.043). Finally, correspondence analysis defined two clusters: HLA-DR4, C4BQ0, Gm(1, 3, 17 23 5*, 21, 28) and BfF associated with stage II, and HLA-DR3, C4AQ0, κm(1) and Gm(3 23 5*) associated with stage I.

These data further support the hypothesis that sarcoidosis results from an interplay of environmental factors and genes, each contributing to the susceptibility/resistance to and/or the clinical heterogeneity of the disease. In addition, these data provide the first evidence of an interaction between immunoglobulin G heavy chain/κ light chain markers and histocompatibility leukocyte antigen class III genes in a disease.

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*Laboratorio HLA, Servizio di Immunoematologia e Trasfusione, ***Unità di Statistica, #Laboratorio di Biochemica e Genetica della Clinica di Malattie dell′Apparato Respiratorio, and ++Dipartimento di Genetica e Microbiologia, IRCCS Policlinico San Matteo and Università degli Studi, Pavia, Italy. ‡Dipartimento di Medicina Clinica e Sperimentale, Immunologia Clinica, Università degli Studi di Padova, and Azienda Ospedale di Padova, Padua, Italy. **Unité de Phy-siopathologie Cellulaire et Moléculaire; CNRS, Hopital Purpan, Toulouse, France.

Correspondence: M. Luisetti, Laboratorio di Biochimica e Genetica, Clinica di Malattie dell′Apparato Respiratorio, Università di Pavia, IRCCS Policlinico San Matteo, *via* Taramelli 5, 27100 Pavia, Italy. Fax: 39 0382502269

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Sarcoidosis is a multisystem granulomatous disorder characterized by noncaseating granulomata and an accumulation of immunocompetent cells at sites of disease activity [1, 2]. Although the aetiology of the disease has not yet been elucidated, there are no reasons to doubt that in sarcoidosis, as in other complex traits, environmental factors may contribute to the onset of the disorder in genetically predisposed individuals [3, 4].

A role for genetic factors in the pathogenesis of sarcoidosis is suggested by the varying incidence of the disease among different ethnic groups, and, more importantly, the occurrence of familial clustering of sarcoidosis [5, 6]. Furthermore, genetic factors are considered to be important in determining the pattern of the disease, its severity, and prognosis: in other words, it is believed that genetic heterogeneity may underlie the different phenotypes of the disease [2, 6].

It is unlikely that a "sarcoidosis gene" exists, but the immunological basis of the disease has prompted researchers to investigate the involvement of histocompatibility leukocyte antigen (HLA), and differing associations with HLA genes have been repeatedly reported (for review, see [6]). It should be mentioned that genetic predisposition to diseases such as sarcoidosis, exhibiting strong pleomorphism and an immune component, must be studied from a multigenic point of view in order to increase the possibility of defining specific predisposing or protective genes.

The first aim of the present study was to investigate immunoglobulin G heavy chain (Gm) and κ light chain (κm) marker polymorphisms in 107 Italian patients affected by sarcoidosis, well characterized for the HLA class I, II and III (C4A, C4B and Bf) polymorphic variants [7–9]. The Gm and κm gene clusters are located on the long arm of chromosomes 14 and 2, respectively and encode specific markers of the constant region of the immunoglobulin G (IgG) heavy (IGHG) and κ light chains, respectively [10, 11]. Together with HLA and T-cell receptor (TCR) genes, Gm and κm allotypes account for the most informative triad of genes involved in immune-related diseases, but so far have not been extensively studied in sarcoidosis. GRUNEWALD *et al*. [12] reported a positive association between the TCR variable region α2.3+, in CD4+ lung T-cells and the HLA-DR3(17),DQ2 haplotype in sarcoidosis.

The second aim of the work was to look at possible interactions between HLA and Gm/κm genes, according to the above-mentioned multigenic hypothesis. The importance of HLA and immunoglobulin gene interactions in enhancing susceptibility or resistance to a great number of pathological conditions [13] has recently been emphasized but, to the authors' knowledge, this study is the first attempt to verify this possibility in sarcoidosis.

Finally, employing the method of correspondence analysis [14], all the closest associations found between the genetic and clinical variants are presented in a single scatterplot. Although this method cannot be used in isolation to rigorously prove or disprove a positive or negative gene/disease association, the bidimensional figure resulting from the analysis may help physicians to gain a better understanding of the relationship between genetic markers and clinical phenotypes. This statistical method was validated in a collaborative study performed in two European countries in which the HLA/sarcoidosis association was considered [9].

Methods

Patients and controls

One hundred and seven unrelated Italian patients, all living in two north-eastern regions of Italy (Lombardy and Veneto), and of Caucasian ancestry, affected by biopsy-proven sarcoidosis were enrolled into this study. According to previous investigations, this series of subjects was clinically characterized in terms of sex, age at disease onset, radiographic stage, extrapulmonary spread and clinical course of the disease (table 1), as well as of a full HLA class I, II, and III (C4A, C4B and Bf) serological typing [8, 9]. Two hundred and seventy-seven blood donors from the same geographical area and of the same ethnic origin as the patients were used as a control group. Their complete HLA class I, II and III typing has been reported previously [15].

Table 1. – Characteristics of sarcoid patients

Patients n	107
Female/Male n	64/43
Age yrs	36±12
Onset n (%)	
Asymptomatic*	37 (3.5)
Symptomatic[+]	70 (65)
Smoking n (%)	
Smokers	33 (31)
Nonsmokers	74 (69)
Stage[#] n (%)	
0	4 (4)
I	38 (36)
II	40 (37)
III	25 (23)
Extrapulmonary spread[‡] n (%)	38 (36)
Outcome[†]	
Good[§]	51 (59)
Poor**	36 (41)
Duration of disease months[†]	39±26

Data are presented as absolute numbers with percentage in parentheses or mean±SD (n=107). *: routine chest radiography; [+]: dyspnoea, fever, erythema nodosum, arthralgia, cough, chest pain, ophthalmic symptoms; [#]: assessed by chest radiography or, when available, high-resolution computed tomographic scan and judged blindly by three different clinicians; [‡]: includes skin, peripheral lymph node, eye, liver, spleen, heart, joints, parotid gland, bone marrow and retroperitoneal lymph node; [†]: n=87; [§]: spontaneous or prednisone-induced resolution; **: prednisone resistance or relapse.

Gm and κm typing

Gm and κm serotyping was performed on serum samples from patients and controls according to the standard procedure of inhibition of agglutination adapted for microtitre or opaline plates [16, 17]. Control samples of known phenotypes (positive and negative for the allotype under study) were typed in parallel with test samples, and appropriate system controls were employed to detect false-positive or false-negative agglutination. The Gm and κm allotypes checked are reported in table 2.

Statistical analysis

The statistical evaluation included the Chi-squared test and Fisher's exact test as appropriate. The odds ratio (OR) was calculated to assess the risk associated with a specific marker. All tests were two-sided with a significance level of 0.05; p-values were corrected for the number of phenotypes or alleles when one locus was considered alone and number of specificity crossings when two genetic systems were considered simultaneously. HLA/Gm and HLA/κm epistatic interactions were analysed as described elsewhere [18, 19]. Epistasis, also called gene interaction, is a mechanism by which a certain genotype confers susceptibility/resistance to a degree dictated by the presence of other genotypes and reflects the interactive effects of mutations, genotypes and/or their biological products. Correspondence analysis [9, 20–22] was employed to cluster the genetic markers and variants in order to visualize all the strongest associations found more easily. The main information drawn from this statistical test was: 1) the phenotypic markers located near the origin of the axes do not account for the variability of the disease; 2) points far from the origin of the axes are of importance; and 3) the distance between markers and clinical features is proportional to their mutual relationship. Correspondence analysis was performed using the SIMCA2 statistical package, implemented in a stand-alone manner in a personal computer environment [23].

Results

Gm and κm polymorphisms

"Protective" Gm phenotypes were observed in patients with respect to controls and inside the clinical phenotypes of the disease (table 2). Gm(3 5*) was present in 3.6% of controls and 0.9% of patients (χ^2=8.31, p=0.0039, p-value for number of specificities tested (p_C=0.05, OR=0.15). The Gm(3 23 5*) phenotype was underrepresented in patients with advanced radiological stages II and III with respect to stages 0 and I ($\chi^2_{(2df)}$=17.61, p=0.00015,

Table 2. – Gm and κm phenotypes in sarcoid and control subjects

	Control	Sarcoidosis										
		Total	Sex		Age at onset		Radiographic stage			Spread		Outcome	
			M	F	<36 yrs	>36 yrs	0+I	II	III	Yes	No	Good*	Poor**
Subjects n	277	107	43	64	56	51	42	40	25	38	69	51	36
Gm phenotypes													
3 23 5*	63.2	50	46.5	53.5	51.8	48	68.3	30	52	45.9	52.2	52.6	45.4
3 5*	3.6	0.9	1.8	0	1.8	0	0	0	4	0	1.4	0	3
1,3,17 23 5*,21,28	13	17	15.3	18.7	12.5	22	12.2	22.5	16	18.9	15.9	14	18.2
1,3,17 5*,21,28	5.8	8.5	12.5	4.5	8.9	8	4.9	15	4	13.5	5.8	7	15.1
1,2,3,17 23 5*,21,28	5.8	3.8	4.3	3.3	3.6	4	0	7.5	4	5.4	2.9	5.3	3
1,2,3,17 5*,21,28	1.1	2.8	0	5.6	3.6	2	0	5	4	0	4.3	1.7	3
1,17 21,28	2.2	3.8	6	1.6	7.1	0	2.4	5	4	2.7	4.3	1.7	6.1
1,2,17 21,28	0.7	0.9	0	1.8	0	2	0	2.5	0	0	1.4	1.7	0
1,3,17 23 5*	2.9	4.7	3.1	6.3	5.4	4	7.3	2.5	4	2.7	5.8	7	3
1,3,17 5*	0.4	0.9	0	1.8	0	2	0	2.5	0	2.7	0	1.7	0
1,17 5*	0	1.9	0	3.8	1.8	2	0	2.5	4	2.7	1.4	1.7	0
1,17 10,11,13,15,16,21,28	0	0.9	0	1.8	0	2	0	2.5	0	2.7	0	0	0
Others	0	3.8	2	5.6	2.6	4	4.9	2.5	4	2.7	4.3	5.3	3
km phenotypes													
(1)	13.3	18.9	25.2	12.6	23.2	14	21.9	17.5	16	16.2	20.3	22.8	15.2
(2)	86.7	81.1	74.8	87.4	76.8	86	78.1	82.5	84	83.8	79.7	77.2	84.8

Data are expressed as a percentage of the absolute value for each category. M: male; F: female; *: spontaneous or prednisone-induced resolution; **: relapse or prednisone resistance. 5*: 5,10,11,13,14.

p_c=0.0058). All other deviations had varying χ^2, but in all cases the p_c was not significant (table 2).

There are only two κm phenotypes definable by serology: κm(1), which comprises the homozygous and heterozygous genotypes κm*1/κm*1 and κm*1/κm*3, indistinguishable from each other apart from by familial pedigree, and κm(3), which represents the homozygous condition κm*3/κm*3. The distribution of these allotypes was not significantly different in patients with respect to the controls and minimal variations were observed even in the different subgroups of patients (table 2).

Histocompatibility leukocyte antigen and Gm/κm

This analysis was commenced by considering all the possible combinations of HLA class II genes (obtained from the authors' previous investigations) and Gm phenotypes in controls, patients and the different disease heterogeneities (table 3). Although no significant interactions were found, combinations of some interest were noted: for instance when the HLA-DR1 and Gm(3 23 5*) phenotypes were present together (1% sarcoidosis *versus* 4.8% controls), the OR decreased from 0.58, ascribable to Gm(3 23 5*) alone, and from the 0.45 of HLA-DR1 alone, to 0.21 when both were present (table 3). Patients with both HLA-DR3 and Gm(3 23 5*) phenotypes were more frequent in radiological stage I, *i.e.* the frequency of this combination was 15% in stage I, 6.8% in stage II and 0% in stage III ($\chi^2_{(2df)}$=6.36, p=0.042, p_c=NS) (table 3).

Subsequently, all possible combinations of C4 alleles and Gm phenotypes were considered (table 3). Despite this, no significant combinations were found, although two interesting "protective" phenotypes, as suggested by impressive OR, were observed: 1) the combination C4A3/Gm(3 5*) was underrepresented in sarcoidosis with respect to controls (0.6 *versus* 6.74%, χ^2=8.65, p=0.0033, p_c=NS) with an OR of 0.09 as against the OR of 0.94 conferred by C4A3 alone, and the OR of 0.15 for Gm (3;...;5*) alone; and 2) the combination C4B1/Gm(3 5*) was also protective, being present at a frequency of 1.2% in patients and of 6.18% in controls (χ^2=5.64, p=0.0176, p_c=NS) with an OR=0.19. The allele C4B1 was not protective *per se* as its OR was 1.14 (table 3).

Thirdly, all possible combinations of Bf alleles and Gm phenotypes were calculated (table 3). This study gave the most interesting epistatic result, since the combination BfS/Gm(3 23 5*) was very poorly associated with radiological stage II with respect to the other stages ($\chi^2_{(2df)}$ =13.86, p=0.00098, p_c=0.043). Another interesting, but nonsignificant, combination was BfS/Gm(3 5*), since its frequency was 0.6% in patients *versus* 5.7% in controls (χ^2=6.77, p=0.0093, p_c=NS OR=0.1). The OR assigned to BfS alone was 0.74 and that ascribed to Gm(3 5*) alone was 0.15. Copresence further enhanced their respective protective effects.

Finally, the same analyses were carried out for HLA and κm genes. No significant combinations were found. However, the combination κm(3)/C4BQ0 was slightly associated with female sex and radiological stage II (χ^2=54.1, p=0.043, p_c=NS, and χ^2=7.86, p=0.019, p_c=NS, respectively (table 3).

Correspondence analysis

This methodology was used, first of all, to analyse the HLA supratype distribution in sarcoidosis in the absence of nuclear families. The term "supratype" refers to a fine definition of the HLA haplotype, including HLA class III genes in addition to the classical class I and II genes [24]. Two major gene clusters were found corresponding to two extended haplotypes associated with opposite clinical behaviours: 1) the supratype HLA-B8,C4AQ0,DR3,DQ2

Table 3. – Histocompatibility leukocyte antigen, Gm and κm interactions in sarcoidosis

	Interactive effect								Single effect					
Interaction	Group 1 %	Group 2 %	Group 3 %	χ^2	χ^2(2df)	p-value	pc-value	OR	Marker	χ^2	χ^2(2df)	p-value	pc-value	OR
Patients *versus* controls														
Gm(3 5*)/BfS	0.6*	5.7**		6.77		0.0093	NS	0.1	Gm(3 23 5*)	5.53		0.0187	NS	0.58
Gm(3 5*)/C4A3	0.6*	6.74**		8.65		0.0033	NS	0.09	Gm(3 5*)	8.31		0.0039	0.05	0.15
Gm(3 5*)/C4B1	1.2*	6.18**		5.64		0.0176	NS	0.19	BfS	1.41		0.23	NS	0.74
Gm(3 23 5*)/DR1	1*	4.8**		4.99		0.051	NS	0.21	C4B1	0.23		0.63	NS	1.14
									C4A3	0.06		0.8	NS	0.94
									DR1	2.99		0.083	NS	0.45
Males *versus* females														
Gm(1,2,17 23 5*,21,28)/BfS	6^{+}	17^{++}		4.26		0.039	NS	0.31	Gm(1,3,17 23 5*,21,28)	0.15		0.69	NS	0.81
Gm(1,3,17 5*,21,28)/BfS	13.6^{+}	4.2^{++}		4.57		0.033	NS	3.55	Gm(1,3,17 5*,21,28)	5.46		0.019	NS	3.88
Gm(1,3,17 5*,21,28)/C4A3	11.8^{+}	2.1^{++}		6.33		0.012	NS	6.13	κm(3)	1.48		0.22	NS	0.61
κm(3)/C4BQ0	2.8^{+}	11.7^{++}		4.1		0.043	NS	0.23	C4A3	0.23		0.63	NS	0.51
Gm(1,3,17 23 5*,21,28)/DR4	0^{+}	3.3^{++}		6.38		0.011	NS	ND	C4BQ0	1.5		0.22	NS	0.51
									DR4	0.34		0.558	NS	0.66
Radiographic stage														
Gm(3 23 5*)/C4A3	$45.4^{\#}$	$25^{\#\#}$	$50^{\#\#\#}$		7.94	0.019	NS		Gm(3 23 5*)		17.61	0.00015	0.0058	
Gm(1,3,17 5*,21,28)/C4A3	$1.5^{\#}$	$15^{\#\#}$	$0^{\#\#\#}$		12.91	0.0016	NS		Gm(1,3,17 23 5*,21,28)		5.88	0.053	NS	
Gm(3 23 5*)/C4B1	$57.5^{\#}$	$30^{\#\#}$	$50^{\#\#\#}$		9.98	0.0068	NS		Gm(1,3,17 5*,21,28)		7.96	0.019	NS	
κm(3)/C4BQ0	$4.5^{\#}$	$16.6^{\#\#}$	$2.7^{\#\#\#}$		7.86	0.019	NS		Gm(1,2,3,17 23 5*,21,28)		6.73	0.035	NS	
Gm(3 23 5*)/BfS	$51.5^{\#}$	$20^{\#\#}$	$44.4^{\#\#\#}$		13.86	0.00098	0.043		C4A3		6.75	0.034	NS	
Gm(1,3,17 23 5*,21,28)/BfF	$0^{\#}$	$10^{\#\#}$	$2.7^{\#\#\#}$		7.69	0.021	NS		C4B1		3.58	0.167	NS	
Gm(1,2,3,17 23 5*,21,28)/BfS	$0^{\#}$	$10^{\#\#}$	$2.7^{\#\#\#}$		7.69	0.021	NS		C4BQ0		5.47	0.065	NS	
Gm(3 23 5*)/DR3	$15^{\#}$	$6.8^{\#\#}$	$0^{\#\#\#}$		6.36	0.0416	NS		BfS		1.11	0.574	NS	
									BfF		1.78	0.41	NS	
									κm(3)		0.68	0.71	NS	
									DR3		8.46	0.014	NS	
Extrapulmonary spread														
Gm(1,3,17 23 5*,21,28)/C4BQ0	$9.6^{\S}$	$1.8^{\S\S}$		5.19		0.023	NS	5.74	Gm(3 23 5*)	2.06		0.15	NS	0.62
Gm(3 23 5*)/C4A3	$25^{\S}$	$45.5^{\S\S}$		6.22		0.013	NS	0.4	Gm(1,3,17 23 5*,21,28)	1.8		0.18	NS	1.76
									C4BQ0	1.82		0.18	NS	1.99
									C4A3	1.3		0.25	NS	0.66
Age at onset														
Gm(1,3,17 23 5*,21,28)/BfS	$6.9^{\ddagger}$	$18.9^{\ddagger\ddagger}$		5.19		0.023	NS	0.32	Gm(1,3,17 23 5*,21,28)	4.1		0.043	NS	0.42
									BfS	0.21		0.65	NS	0.85
Outcome														
Gm(1,3,17 5*,21,28)/C4B1	$3.9^{\dagger}$	$13.6^{\dagger\dagger}$		4.29		0.038	NS	0.26	Gm(1,3,17 5*,21,28)	3.89		0.049	NS	0.31
									C4B1	1.43		0.23	NS	0.6

*: sarcoid; **: control; $^{+}$: males; $^{++}$: females; $^{\#}$: stages 0 and I; $^{\#\#}$: stage II; $^{\#\#\#}$: stage III; §: yes; §§: no; ‡: <36 yrs; ‡‡: >36 yrs; †: good; ††: poor. pc-value: p-value corrected for the number of specificities tested; OR: odds ratio; ND: not determined.

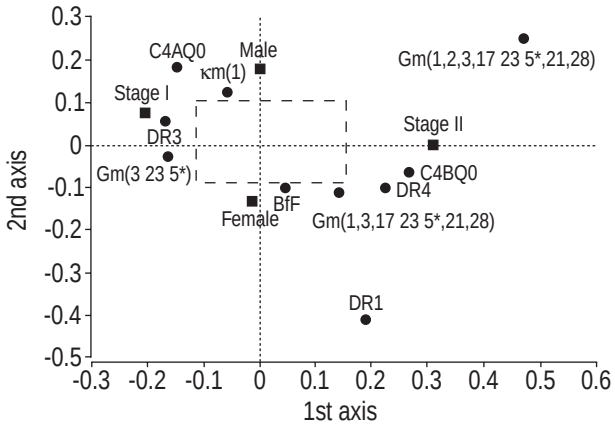

Fig. 1. – Correspondence analysis of sarcoidosis clinical heterogeneity with all of the polymorphisms investigated. The bidimensional figure, generated by plotting the first two axes, accounts for 65.1% (42.1% of the first axis and 23% of the second axis) of the total variability. All variables in the central square (– – –) were not reported, since they were noninformative.

defined the less severe form of sarcoidosis, with delayed onset, radiological stage I and good outcome, either spontaneous or prednisone-induced; and 2) in contrast the supratype HLA-B35,BfF,DR11,DQ7 was associated with the worst form of sarcoidosis characterized by an early onset, advanced radiological stage and poor outcome.

Secondly, three main clusters emerged from the scattergram of the correspondence analysis elaborated taking into account all of the polymorphisms investigated (fig. 1). The most important results revolve around radiological stages I and II and the patients′ sex: 1) HLA-DR4, C4BQ0 and Gm(1,3, 17 23 5*, 21,28) were associated with radiological stage II; 2) HLA-DR3, C4AQ0 and Gm(3 23 5*) characterized radiological stage I; and 3) BfF was peculiar to females and κm(1) was more frequent in males. All of the other variables are of little interest as they were located near the intersection of the axes (dashed central square).

Discussion

A genetic background for sarcoidosis itself and its clinical phenotypes is widely recognized [2, 6, 9, 25, 26]. The authors′ previous investigations have reported the HLA "supratype" distribution in 107 unrelated Italian patients affected by sarcoidosis. In the absence of nuclear families, the supratype study was performed using correspondence analysis. Two major gene clusters were found corresponding to two extended haplotypes, each associated with a different clinical behaviour: 1) supratype HLA-B8, C4AQ0, DR3, DQ2 defined a form of sarcoidosis with delayed onset, radiographic stage I and spontaneous resolution; and 2) supratype HLA-B35, BfF, DR11, DQ7 seemed to be associated with a form of sarcoidosis with early onset, advanced radiographic stage and poor outcome [8, 9].

This study focuses on a particular aspect of the immunogenetics of sarcoidosis. A set of polymorphic genes, so far poorly investigated in sarcoidosis, notwithstanding their role in the pathogenesis of conditions with immune system impairment, were analysed. Namely, the serological allotypic polymorphism of the constant region of the IgG heavy chain (Gm) and that of the constant region of the κ light chain (κm) were studied. Three subclasses of human IgG (IgG1, IgG2 and IgG3) are characterized by 18 different Gm allotypes. Genes encoding heavy chains are closely linked and located on the long arm of chromosome 14 [10], and so are inherited in fixed combinations or haplotypes. In the early 1980s, it was shown that different Gm allotypes (phenotypes and/or haplotypes) affect the immune response to infectious agents, and that of immune-mediated disorders [27–30]. Beyond an early report, in which G3m(5) was found in 94 of 95 black patients with sarcoidosis, but also in 88 of 97 controls [31], immunoglobulin markers have never been exhaustively studied in sarcoidosis. In the present paper, a Gm/κm allotypic association is described in sarcoidosis (table 2). The most consistent finding is that the Gm* 3±23 5* haplotype correlates with a protection factor for the disease.

The Gm and κm information were recorded in the HLA database and a correspondence analysis test performed. In the scattergram, the genetic markers clustered at two main poles, one located in the upper left sector, and the other in the lower right sector (fig. 1). A graphic interpretation of the patterns provides evidence that the genetic variants mainly revolve around radiographic stages I and II. In addition, their distances from these points represent the rate of their genetic specific contribution. Based on this framework, radiographic stage I was related to the various markers according to the following aetiological fraction hierarchy: HLA-DR3 > Gm(3 23 5*) > C4AQ0 > κm(1), whereas radiographic stage II related to C4BQ0 > HLA-DR4> Gm(1,3,17 23 5*,21,28).

Two main questions arise from the present data, including the HLA class II and III analysis. First, the significance of the association between the genes and radiographic stage of the sarcoidosis, which represents the most fluctuating factor in this disease, might be questioned. Secondly, do the immune response genes HLA-DR and C4 act independently of each other, or is it rather a matter of linkage disequilibrium, so commonly seen in the HLA system? In response to the first question, it should be recalled that associations between HLA class I and II alleles and radiological stages have been previously found in patients of different ethnic origins [9]. Concerning the latter question, it is worth mentioning that, in Caucasian populations, the C4A null allele (C4AQ0) is part of the supratype HLA-A1, CW7, B8, SC01, DR3, DQ2, also referred to as ancestral haplotype (AH) 8.1, whereas the C4B null allele (C4BQ0) segregates with the HLA-B44, SC30, DR4, DQ7 supratype, also referred to as AH44.1. AHs are supposed to carry a special gene content which favours their "block segregation" and maintenance in the populations [26]. The "protective" role of HLA-DR3 in conferring a good prognosis has been recently confirmed in another ethnic group of sarcoid patients [24], and the authors of that study speculated as to whether this might be related to its selective capacity to present mycobacterial antigens [32]. However, C4AQ0 and C4BQ0 alleles seem to play their own role in sarcoidosis, independent of that conferred by the HLA-DR3 or -DR4 alleles, as demonstrated by the correspondence analysis (see fig. 1).

The HLA-DR4, C4B, Bf and Gm/κm gene interactions deserve a final mention. In this respect, and in the framework of the putative polygenic determinants of sarcoidosis [2, 4, 6], some interesting synergistic gene effects, such as the protective effect towards stage II conferred by the combination Gm(3 23 5*)/BfS and the protection rate against the disease conferred by the combinations C4A3/Gm(3 5*) and BfS/Gm(3 5*), were observed. There is no doubt that the most intriguing finding from this body of statistical comparisons is that Gm/κm and HLA-class III gene interactions also exist, besides the clinical ones among Gm/κm and HLA-class II genes, which are evident in sarcoidosis, as well as in other immunological disorders. To the authors′ knowledge, this is the first time that these interactions have been found in a disease.

These results should be interpreted taking into account the fact that Gm, κm and C4 genes are involved in the control of the humoral immune response [30]. Although the mechanisms responsible for the role of Gm allotypes regarding the IgG subclass concentration are still unknown, a relationship between low levels of IgG1 and the phenotype Gm(3 23 5*) has recently been reported [30]. The decreased frequency of Gm(3 23 5*) in patients with sarcoidosis is consistent with this evidence. Humoral mechanisms are important in the pathogenesis of granuloma formation and, in sarcoidosis, immunoglobulin levels are often elevated, with increased antibody titres against a variety of antigens [33].

In conclusion, although obtained in a relatively small sample size of defined ethnic origin, thus remaining far from providing a final answer, the present data seem to further support the hypothesis that sarcoidosis is the result of a complex interplay of environmental factors and genes, each contributing, with an independent aetiological fraction, to susceptibility or resistance and/or to the clinical heterogeneity of the disease. These data could prompt investigators in this field to study, at a molecular level, the histocompatibility leukocyte antigen Gm/κm interactions in a larger series of sarcoid patients of different ethnicity. A better identification of the genetic factors involved in sarcoidosis may also be useful in defining the individual background against which environmental causes act.

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