Differences in HLA antigens between patients with mixed connective tissue disease and systemic lupus erythematosus

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Abstract

Patients with mixed connective tissue disease (MCTD, n=32) or systemic lupus erythematosus (SLE, n=60) were typed for HLA-A, B, C, Dw, and DR antigens. All patients with SLE fulfilled at least four criteria of SLE and the patients with MCTD met the criteria proposed by Alarcon-Segovia (1989). The presence of antibodies to Sm was not considered as an exclusion for MCTD. In the patients with SLE, Dw3, DR3, and the associated B8 and A1 antigens were increased, whereas in the patients with MCTD an increased frequency of Dw4 was found (45 v 18% in controls v 14% in SLE). Of the subtypes of DR4, Dw4 was present in all but one of the DR4 positive patients. The frequency of DR4 in patients with MCTD (52%) differed significantly from that of controls (28%). The strong association of MCTD to one DR4 subtype was further seen in the significantly increased frequency of the B15, DR4 combination. Thus the genetic background seems to be different in patients with MCTD from that in patients with SLE. This could partly explain the clinical differences between these diseases.

Mixed connective tissue disease (MCTD) has been suggested to be a distinct clinical entity.¹ Hallmarks of this disease are overlapping clinical signs, the absence of or only mild renal disease, and high titres of antibodies to nuclear ribonucleoprotein antigen (anti-RNP). There has been some disagreement on the differentiation of MCTD from systemic lupus erythematosus (SLE).² There are no specific diagnostic criteria for MCTD and many of the patients could easily be diagnosed as having SLE with anti-RNP. Alarcon-Segovia³ proposed some criteria for MCTD in 1982 and re-evaluated them in 1989,⁴ when antibodies to Sm were accepted in MCTD. MCTD could be a subset of SLE meeting certain criteria for diagnosis and having a typical prognosis, but a classification scheme for this subset is still obscure.⁵

Autoimmune disorders are divided into subgroups by clinical, serological, and genetic factors. Genetic rules can be the most powerful elements determining the type and course of the disease in each patient. The disease associations of the products of the major histocompatibility complex have been studied extensively. HLA-A1, B8, DR2 and/or DR3 are associated with SLE⁶⁻¹⁷ and, interestingly, DR2 and DR3 with some autoantibodies appear in SLE and in other autoimmune diseases.^{5-8 10 14 15 18}

Studies on the HLA associations of MCTD

are few and not very convincing.^{19 20} A couple of reports have linked HLA-DR4 to anti-RNP but not to MCTD itself.^{6 21} In this work we measured HLA antigens in patients with MCTD and SLE to see if genetic differences between these two diseases could be seen.

Materials and methods

PATIENTS AND CONTROLS

We studied a total of 60 patients with SLE and 32 with MCTD. The patients were admitted to three Finnish rheumatology clinics between November 1982 and the end of May 1987 and were examined thoroughly following a pre-determined checklist. The diagnosis of each patient was confirmed by two rheumatologists and was re-evaluated at the end of the study if necessary. For a diagnosis of SLE, at least four of the 1982 revised criteria²² had to be fulfilled; the clinical and laboratory criteria proposed by Alarcon-Segovia³ (table 1) were used for a diagnosis of MCTD. Table 2 lists the characteristics of the patients with MCTD. The presence of antibody to Sm did not exclude MCTD in this study.⁴ The control group consisted of 270 healthy untreated blood donors and laboratory staff.

HLA TYPING

HLA-A, B, and C antigens from 32 patients with MCTD and 60 patients with SLE were determined with a standard microlymphocytotoxicity method.²³ HLA-D typing (22 patients with MCTD and 51 with SLE) was performed with a panel of local homozygous typing cells for Dw1-Dw4 and Dw14 antigens.²⁴ HLA-DR typing of 26 patients with MCTD and 48 with SLE was performed in parallel on three commercial typing trays following the instructions of the manufacturers (One Lambda, Los Angeles, CA, USA; Fresenius, Oberursel, Germany; Behringwerke, Marburg, Germany).

Table 1 Clinical and laboratory criteria for the diagnosis of mixed connective tissue disease, as classified by Alarcon-Segovia³

- 1 Manifestations considered characteristic of two or more connective tissue diseases
- 2 Three or more* of the following signs or symptoms definitely present:
 - Oedema of the hands
 - Raynaud's phenomenon
 - Scleroderma
 - Arthritis
 - Myositis
- 3 Antibodies to RNP at a haemagglutination titre greater than 1:1600, negative or not higher than 1:160 with ribonuclease treated extractable nuclear antigen

*If the first three symptoms are present, one of the other two is also required.

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Table 2 Characteristics of patients with mixed connective tissue disease with high titres of antibodies to extractable nuclear antigens; titre of antibodies to extractable nuclear antigen and double stranded DNA and clinical findings used as criteria for the diagnosis of MCTD

Patient No	Sex	Antibodies to*			Clinical findings†		
		ENA	Sm	dsDNA	Oedema	Acro	Myo
1	F	81 920	0	0	+	+	+
2	F	163 840	0	0	+	—	+
3	M	327 680	0	0	+	+	+
4	F	163 840	0	160	+	—	+
5	F	40 960	0	0	+	+	+
6	F	163 840	0	160	+	—	—
7	F	163 840	0	0	+	—	—
8	F	5 120	0	0	+	+	—
9	F	81 920	0	0	+	+	—
10	F	327 680	0	0	+	+	+
11	F	163 840	0	0	+	+	—
12	F	327 680	0	0	+	+	+
13	F	81 920	0	0	+	—	—
14	F	20 480	0	0	+	—	—
15	F	163 840	0	0	+	—	—
16	F	40 960	0	0	+	—	—
17	F	81 920	0	0	+	—	—
18	F	327 680	0	40	+	+	+
19	F	81 920	0	0	+	—	—
20	F	163 840	0	0	+	+	—
21	F	163 840	0	0	+	—	—
22	F	163 840	81 920	1 280	—	+	+
23	M	163 840	2 560	0	+	—	—
24	F	327 680	81 920	10 240	—	—	+
25	F	163 840	1 280	0	+	—	+
26	F	327 680	0	0	+	—	+
27	F	655 360	0	0	+	—	—
28	M	40 960	0	0	+	—	—
29	F	163 840	0	0	+	—	+
30	M	163 840	0	0	+	—	+
31	M	81 920	0	0	+	—	—
32	F	81 920	0	0	+	—	+

*The results are expressed as reciprocals of titres. All extractable nuclear antigen haemagglutination positive results were confirmed by the immunodiffusion method. ENA=extractable nuclear antigen; dsDNA=double stranded DNA.

†Oedema=oedema of hands, acro=acrosclerosis and myo=myositis. Raynaud's syndrome was present in all patients and arthritis in all but patient 26.

DETERMINATION OF ANTIBODIES TO EXTRACTABLE NUCLEAR ANTIGEN

Antibodies to extractable nuclear antigen were analysed by passive haemagglutination²⁵ and by immunodiffusion.²⁶ Ammonium sulphate (30–60%) precipitated antigen was elected for

immunodiffusion.²⁷ Extractable nuclear antigen immunodiffusion was calibrated by reference serum samples for antibodies to Sm, RNP, and SSB, kindly supplied by the AF-CDC ANA reference laboratory (Atlanta, GA, USA). Antigen for haemagglutination was prepared by ethanol precipitation.²⁵ Haemagglutination titres after RNase digestion of extractable nuclear antigen were also recorded. If the titre with digested extractable nuclear antigen was two or more dilution steps lower than in the test without digestion, but not zero, the serum sample was considered to contain both Sm and RNP antibodies.

ANTIBODIES TO DOUBLE STRANDED DNA

Antibodies to double stranded DNA were measured by the indirect immunofluorescence method of Aarden *et al.*²⁸

STATISTICAL ANALYSIS

Statistical analysis of the frequencies of the HLA antigens was performed by χ^2 or Fisher's exact test. For comparison, the frequencies in healthy subjects partly published by Partanen *et al.*²⁹ were applied. Corrected p values were obtained for the differences in HLA frequencies by multiplying the p values from these tests with the number of specificities tested for in the relevant HLA locus.

Results

Table 3 shows the frequencies of all DR and Dw antigens determined; the A, B, and C specificities which differed between the groups are indicated.

Table 3 Frequencies of the HLA-A, HLA-B and HLA-C antigens which differed between groups and of all typed HLA-Dw and HLA-DR antigens

	Controls		Patients with MCTD		Patients with SLE		RR	
	n	(%)	n	(%)	n	(%)	MCTD	SLE
Group 1*								
HLA-A1	40	(15)	5	(16)	17	(28)†	1.85	2.27
HLA-A3	128	(47)	20	(63)†*	20	(33)		0.55
HLA-Aw19	30/268	(11)	5	(16)	14	(23)†		2.41
HLA-B8	39	(14)	6	(19)†*	27	(45)§	3.50	4.85
HLA-B16	20	(7)	7	(22)†*	5	(8)		0.33
HLA-B35¶	78	(29)	12	(38)†*	7	(12)†		
HLA-Cw4	68/265	(26)	12	(38)†*	10	(17)		
Group 2*								
HLA-Dw1	70	(36)	5	(23)	8	(16)‡	0.51	0.32
HLA-Dw2	45	(23)	3	(14)	10	(20)		
HLA-Dw3	23	(12)	5	(23)	25	(49)§	3.87	7.07
HLA-Dw4	34	(18)	10	(45)‡*	7	(14)		0.74
HLA-Dw14	16/119	(13)	2	(9)	7	(14)		
Group 3*								
HLA-DR1	60/193	(31)	5	(19)	8	(17)	0.53	0.44
HLA-DR2	46/194	(24)	4	(15)	11	(23)		
HLA-DR3	31/192	(16)	6	(23)†*	25	(52)§		5.65
HLA-DR4	54/191	(28)	13	(52)†*	11	(23)		
HLA-DR5	18/190	(9)	1	(4)	3	(6)		
HLA-DRw6	42/194	(22)	5	(19)	9	(19)		
HLA-DR7	24/192	(13)	0	(0)	2	(4)		
HLA-DRw8	34/192	(18)	8	(31)	11	(23)		

Abbreviations: MCTD=mixed connective tissue disease; SLE=systemic lupus erythematosus; RR=relative risk.

*Number of subjects: Group 1, controls 270, MCTD 32, SLE 60; Group 2, controls 192, MCTD 22, SLE 51; Group 3, controls 192, MCTD 26, SLE 48.

†p<0.05.

‡p<0.01.

§p<0.001.

||n for MCTD=25.

¶n for SLE=59.

*Differences between MCTD and SLE.

†Differences between MCTD and controls.

Table 4 Relative risks of some HLA combinations to mixed connective tissue disease and systemic lupus erythematosus. Results expressed as number positive/total number of subjects

HLA antigen combination	Controls (n)	MCTD			SLE		
		n	p value	RR	n	p value	RR
A1, B8, Dw3	10/179	3/22	NS	2.67	13/51	<0.001	5.78
B8, Dw3	18/179	4/22	NS	1.99	22/51	<0.001	6.79
B8, DR3	26/191	4/26	NS	1.15	22/48	<0.001	5.37
B15, Dw4	15/178	6/22	0.019	4.08	5/51	NS	1.18
B15, DR4	17/189	7/25	0.013	3.93	5/48	NS	1.18
B35, Dw1	42/179	5/22	NS	0.96	4/50	0.027	0.28
B8, B27	1/270	0/32	NS	*	4/60	0.002	19.21
Dw4, DR4	21/130	10/20	0.001	5.19	6/43	NS	0.84

Abbreviations: MCTD=mixed connective tissue disease; SLE=systemic lupus erythematosus; RR=relative risk; NS=not significant.

*Number of mixed connective tissue disease patients with this combination=0.

In patients with SLE Dw3 and DR3 were increased with the highest relative risks. Of the antigens associated with these two, B8 was increased very significantly, but the increase in the A1 antigen was less obvious. An increase in Aw19 and a decrease in B35 and Dw1 were also seen.

The group of patients with MCTD clearly differed from those with SLE. The frequencies of DR3 and associated antigens were similar in the control subjects. The HLA-Dw4 antigen was increased, and the frequencies of DR4 and B16 differed significantly from those of control subjects. Ten of the 11 DR4 positive patients with MCTD had Dw4, but only one had Dw14, which is the second MLC defined specificity associated with DR4 which is frequent in Finland. The overall frequency of Dw14 was the same in patients with MCTD and SLE and in control subjects. The association of MCTD with the Dw4 subset of DR4 is also seen in table 4, in which risks for antigen combinations are presented. The frequencies of several other HLA antigens differed significantly between patients with MCTD and those with SLE: A3, B35, Cw4, Dw4, and DR4 were increased and B8 and DR3 were decreased in patients with MCTD compared with those with SLE.

Discussion

In this work we analysed types of HLA antigens in 32 patients with MCTD and 60 with SLE to answer two questions: (1) are there any HLA associations with MCTD, and (2) are there genetic differences between patients with MCTD and SLE? The answer to both questions was positive. The frequencies of HLA antigens in MCTD were similar to those in control subjects, with the exception of an increased frequency of HLA-Dw4 (10/22 (45%) in patients with MCTD, 34/192 (18%) in control subjects, uncorrected $p=0.006$). The frequencies of DR4 and B16 were also slightly increased ($p<0.05$). In both control subjects and patients with SLE the frequency of the Dw4 subtype of DR4 was much lower than it was in patients with MCTD, half of whom carried Dw4.

Previously well known HLA associations with SLE were also seen here. A1, B8, Dw3, and DR3 were increased.^{6-9 11 12 16 17} Dw2 and DR2 frequencies in our patients with SLE were lower than reported previously^{6 10 13-17} and also

lower than in our controls. It has been proposed that both DR2 and DR3 antigens might be markers for immune hyper-responsiveness, i.e. increased seroreactivity (anti-Ro, anti-DNA, RF, and hyperglobulinaemia),¹⁵ thus our patients with SLE would have an antibody pattern different from, for example, American or Japanese series.¹³ Other differences between patients with SLE and control subjects were an increase in the frequency of Aw19, also reported by Stastny,³⁰ and decreases in B35, Dw1, and DR1; these differences were not significant after the correction of p values. The decreases in B35, Dw1, and DR1 antigens (which are very common and occur in one of the most frequent haplotypes in the Finnish population) may be secondary to the increase of the other antigens. Within the well known haplotype A1, B8, Dw3 the highest relative risk for Dw3 supports the proposal that the susceptibility factor to SLE resides in the D end of the HLA region. We could also confirm the previous report³¹ that the combination of B8 and B27 antigens is increased in patients with SLE (relative risk, 19.21), but only a few patients carried this combination.

The association to the Dw4 subtype of DR4 separates patients with MCTD from those with SLE and even from those with rheumatoid arthritis. The Dw4 to Dw14 ratio in patients with MCTD was 10:2 in the whole series (table 3) and 10:1 in DR4 positive patients; the respective values for patients with SLE were 7:7 and 6:5. The increase of the B15, DR4 (Dw4) combination suggests that the susceptibility genes are enriched in one particular haplotype. The two best known disease associations of DR4 (insulin dependent diabetes and rheumatoid arthritis) differ from each other with respect to subtypes. The association of rheumatoid arthritis with DR4 is well documented,³² but the proportion of Dw14 is enhanced.^{33 34} In insulin dependent diabetes, the B15, C4A3B3, Dw4, DR4 haplotype gives an excessive relative risk to the disease, although only a fraction of the patients actually have this whole haplotype.³⁵ It appears that MCTD genetically resembles rheumatoid arthritis and insulin dependent diabetes mellitus more closely than it resembles SLE, which is surprising because the opposite situation is seen clinically. We conclude from these and previously published²⁰ results that the susceptibility to MCTD is linked with Dw4. The primary association of HLA antigens might not be with the clinical syndromes themselves but with the autoantibodies found.^{6 8 21} The autoantibodies suggested to be associated with HLA include antibodies to SSA (DR3) and RNP (DR4). The association of DR4 with anti-RNP has been reported to be very strong, and anti-RNP positive patients with or without MCTD had the same frequency of HLA-DR4.²¹ We are now trying to determine if the primary association of HLA-Dw4 is with anti-RNP or clinical MCTD.

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