HLA antigens in Italian patients with primary Sjögren's syndrome

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SUMMARY Twenty eight Italian patients with primary Sjögren's syndrome were typed for class I and class II alloantigens of the major histocompatibility complex. Patients with Sjögren's syndrome had higher prevalence of DR3 (46.4% v 14% in the control population, p corrected <0.02), while similar prevalence was found for DR2 and DRw52 alloantigens. DR3 correlated with DRw52 ($p<0.0001$), anti-Ro(SSA) ($p<0.0002$) and anti-La(SSB) ($p<0.02$) antibodies, and extraglandular manifestations ($p<0.02$). In addition, extraglandular involvement was associated with anti-Ro antibodies ($p<0.05$) and raised gammaglobulins ($p<0.02$). In Italian patients with primary Sjögren's syndrome DR3 is the genetic marker related to this clinical entity and seems to identify a disease subset characterised by autoantibody production and extraglandular manifestations.

Key words: histocompatibility testing, collagen diseases, antinuclear antibodies, gammaglobulins, rheumatoid factor.

Sjögren's syndrome (SS) has been defined as an autoimmune exocrinopathy, characterised by polyclonal B cell proliferation, with infiltration in exocrine glands and extraglandular organs, and production of multiple autoantibodies, some of which are antibodies to small molecular weight ribonucleoproteins, named Ro(SSA) and La(SSB).¹ SS may occur in association with other connective tissue diseases or immunological disorders (secondary SS), or as an isolated clinical entity (primary SS).¹ Major histocompatibility complex antigens (HLA system) appear to have an important genetic influence on the development of SS.²⁻⁶ While HLA-DR3 antigen has been reported to be associated with primary SS, and DR4 with the secondary form of the syndrome, another HLA specificity located on DR molecules, now defined as DRw52, is known to be associated with both primary and secondary SS.^{5,6} Furthermore, some DR alloantigens, specifically DR3 and DR2, seem to mediate the expression of anti-Ro and anti-La antibodies in patients with primary and secondary SS,⁶ and in patients with systemic lupus erythematosus.^{7,8}

Since it is known that genetic background^{9,10} and clinical expression^{11,12} of autoimmune diseases may change in different ethnical groups, in the present work we have studied the HLA profile and the clinical and serological features in an unselected group of Italian patients with primary SS.

Patients and methods

PATIENTS

The study included 28 patients with primary SS, referred to the Rheumatic Disease Unit of the University of Pisa. The diagnosis of primary SS was based on the clinical symptoms of dry eyes and dry mouth, confirmed by objective demonstration of xerophthalmia (positive Schirmer's test) or keratoconjunctivitis sicca (positive rose bengal staining), or both, and on the presence of salivary glands involvement (positive lip biopsy or hydrostatic sialography, or both), in the absence of other associated diseases.¹

CLINICAL EVALUATION

To evaluate the presence of symptoms and signs of extraglandular involvement each patient was followed up for a time ranging from one to nine years.

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In the present study patients were considered to have extraglandular involvement when at least one of the following clinical features was present: (a) leucopenia or thrombocytopenia, or both; (b) radiological or functional evidence of lung involvement;¹³ (c) cutaneous involvement, i.e., cutaneous vasculitis or hypergammaglobulinaemic purpura, confirmed by cutaneous biopsy; (d) Raynaud's phenomenon; (e) articular manifestations, i.e., arthralgias or non-erosive, non-deforming arthritis; (f) autoimmune thyroiditis; (g) peripheral neuropathy (confirmed by electromyography); (h) lymph node or spleen enlargement, or both.

SEROLOGICAL STUDIES

Rheumatoid factor (RF) was determined by the agglutination of latex particles coated with aggregated Cohn human fraction II by the microslide technique (Behringwerk, Marburg, West Germany). In this study a positive RF test was defined when a titre $\geq 1/40$ was present at least twice in the patient's course. Similarly, hypergammaglobulinaemia was defined when serum gammaglobulin levels were ≥ 1.8 g/dl, a value clearly exceeding the normal range (0.7–1.7 g/dl) established in our laboratory.

Sera from all the patients were also examined for antibodies to Ro, La, RNP, and Sm by counterimmunoelectrophoresis, according to Bunn *et al*¹⁴, with extracts of human spleen¹⁵ and calf thymus¹⁶ as sources of antigens. Reference sera were kindly provided by Dr M Reichlin and Dr G R V Hughes. In patients showing negativity for these autoantibodies in the first serum sample the determination was repeated in at least one other serum sample withdrawn from the patients at a different follow up time.

HLA TYPING

Typing for class I antigens was performed with the standard microlymphocytotoxicity method of the National Institute of Health.¹⁷ Typing sera were supplied by the Italian Committee for Tissue Typing and selected from among those tested at the 8th International Workshop. Three hundred and twenty two ethnically homogeneous, genetically unrelated subjects typed with the same sera during the same period served as control group.

Class II antigens were studied by commercial trays (UCLA Tissue Typing Laboratory, Los Angeles, Ca). A control group of 62 subjects was tested with the same method. HLA designations were named according to the 9th Histocompatibility Workshop nomenclature. The following HLA antigens were determined:

Class I:

Locus A: A1, A2, A3, A9, A10, A11, A23(9), A24(9), A25(10), A26(10), A28, A29(w19), A30(w19), A31(w19), A32(w19), A33(w19), Aw68(28), Aw69(28).

Locus B: B5, B7, B8, B12, B13, B14, B15, B16, B17, B18, B21, Bw22, B27, B35, B37, B38(16), B40, Bw41, Bw44(12), B49(21), B51(5), Bw55(w22), Bw63(15), Bw4, Bw6.

Class II:

DR specificities: DR1, DR2, DR3, DR4, DR5, DRw6, DR7, DRw8, DRw9, DRw10, DRw52, DRw53.

DQ specificities: DQw1, DQw2, DQw3.

As there is a considerable overlap in nomenclature it may be helpful to clarify the identity of some DR and DQ specificities with respect to the previous designations: DRw52=MT2, DRw53=MT3, DQw1=DC1=MB1=MT1, DQw2=MB2=DC3, DQw3=MB3=MT4=DC4.

STATISTICAL ANALYSIS

Comparisons of the frequencies of HLA antigens between patients and controls were made by generating 2x2 tables with the χ^2 method with Yates's correction; the corresponding p values were corrected by multiplication by the number of tested A, B, DR, or DQ antigens.

Comparisons between HLA specificities and clinical-serological features of patients were made by a two tailed Fisher's exact test.

Results

Age, sex, disease duration, DR and DQ typing, and the main clinical and serological findings of our patients with primary SS are reported in Table 1.

Anti-Ro and anti-La antibodies were present in respectively 15/28 (53.6%) and 8/28 (28.6%) cases. It is noticeable that anti-La antibodies were always associated with the presence of anti-Ro, while in seven cases anti-Ro antibodies were detected alone. Raised gammaglobulin levels and RF were found in a high proportion of patients (17/28 (61%) and 19/28 (68%) respectively), and extraglandular involvement (68.9%), Raynaud's phenomenon, and articular involvement were the most frequent extraglandular manifestations.

None of the tested HLA-A and -B specificities showed a statistically significant prevalence in patients with respect to the control group, though B8 prevalence was higher in the SS group (21.4%) than in the controls (13.3%).

In contrast, among the DR and DQ alloantigens DR3 was significantly more frequent in patients (46.4%) than in controls (14%), ($p < 0.002$, corrected p , $p_c < 0.02$). DRw52 was strictly associated with the presence of DR3 ($p < 0.0001$), but its

prevalence in patients and controls was the same.

When clinical and serological features in patients with or without DRw52 and DR3 alloantigens were compared (Table 2) both these DR specificities were closely linked to the presence of anti-Ro antibodies

Table 1 Sex, age, disease duration, DR and DQ alloantigens, clinical and serological findings of 28 patients with primary Sjögren's syndrome

No	Sex	Age (years)	Disease duration (years)	DR and DQ alloantigens	Anti-Ro/La antibodies	RF	Raised gamma-globulins	Extraglandular involvement*
1	F	42	9	DR8, DQw3	Ro	—	—	No
2	M	70	8	DR4, DR7, DRw53, DQw3	—	+	—	No
3	F	51	3	DR3, DR5, DRw52, DQw3	Ro	+	+	A, Ray
4	F	54	12	DR3, DRw52	Ro/La	+	+	S, Th, P, Leu
5	F	57	7	DR4, DRw53, DQw3	—	—	—	A, Ray, Va
6	F	49	8	DR2, DR3, DRw52, DRw53	Ro/La	+	+	No
7	F	39	8	DR2, DQw1	—	—	—	No
8	F	34	13	DR2, DQw1	—	+	+	Ray, P, Leu
9	F	42	7	DR6, DRw52	—	+	+	No
10	F	59	5	DR3, DR7, DRw52, DRw53	Ro	—	+	Lu
11	F	43	10	DR5, DR7, DRw53, DQw3	—	+	+	No
12	F	42	6	DR3, DR5, DRw52	Ro	+	+	A, Ray
13	F	54	4	DR3, DRw52, DQw2	Ro/La	+	+	A
14	F	57	15	DR2, DR3, DRw52, DQw1	Ro	+	+	Lu, Leu
15	F	56	36	DR3, DRw52	—	—	—	Ray, P, PN
16	F	44	4	DR7, DRw53	—	+	—	No
17	F	46	6	DR7, DRw53	—	—	—	No
18	F	56	3	DR7, DRw53	—	—	+	Ray, Leu, Va
19	F	18	2	DR2, DR3, DRw52, DQw1	Ro/La	—	+	Lu/Ly
20	F	30	3	DR3, DR5, DRw52	Ro/La	+	+	Ray
21	F	52	11	DR8, DQw3	—	—	—	No
22	F	59	12	DR1, DR3, DRw52, DQw1	Ro/La	+	+	Th, Leu, Thr
23	F	50	3	DR1, DR5, DRw52, DQw3	Ro/La	—	+	Ray, Thr
24	F	53	12	DR3, DR8, DRw52	Ro	+	—	P
25	F	53	15	DR1, DR4, DRw52, DRw53, DQw1	—	+	+	A, Th
26	F	57	4	DR6, DRw52	Ro	+	+	A, Lu, Leu, Thr
27	F	29	18	DR2, DR3, DRw52, DQw1	Ro/La	+	+	Ray, Th
28	M	39	7	DR2, DRw53, DQw1	—	+	+	Lu

*A=arthritis/arthralgias; Ray=Raynaud's phenomenon; S=splenomegaly; Th=thyroid involvement; P=purpura; Va=cutaneous vasculitis; Leu=leucopenia; Thr=thrombocytopenia; Ly=lymph node enlargement; Lu=lung involvement; PN=peripheral neuropathy.

Table 2 Relation between HLA specificities DRw52 and DR3 and serological parameters and extraglandular involvement in 28 patients with primary Sjögren's syndrome*

	Anti-Ro antibodies	Anti-La antibodies	RF	Raised gammaglobulins	Extraglandular involvement
DRw52	0.0003	0.0097	0.1245	0.0104	0.0104
DR3	0.0002	0.0108	0.2543	0.1145	0.0157

*The figures in the table are p values obtained by a two tailed Fisher's exact test.

Table 3 Relation of different serological parameters to each other and with extraglandular involvement in 28 patients with primary Sjögren's syndrome*

	Anti-Ro antibodies	Anti-La antibodies	RF	Raised gammaglobulins
Anti-La antibodies	0.0025			
RF	0.4328	0.6692		
Raised gammaglobulins	0.0418	0.0292	0.0346	
Extraglandular involvement	0.0418	0.2144	0.6775	0.0127

*The figures in the table are p values obtained by a two tailed Fisher's exact test.

and extraglandular involvement, while DRw52, but not DR3 alloantigen, was associated with hypergammaglobulinaemia. The presence of RF did not appear to be related either to DRw52 or DR3 specificities. DR2 alloantigen which was present in three cases alone and in four together with DR3, seemed to be not related either to autoantibody presence or extraglandular involvement.

With regard to the clinical-serological relations both hypergammaglobulinaemia and anti-Ro antibodies, but not RF and anti-La, were strictly associated with extraglandular involvement (Table 3).

Discussion

In this study HLA profiles and clinical and serological findings were evaluated in an unselected series of Italian patients with primary SS. Our results showed that: (a) primary SS is strictly associated with DR3 alloantigen; (b) DR3 and DRw52 are associated with extraglandular manifestations of the disease and with certain serological markers, i.e., anti-Ro/La antibodies; (c) finally, DRw52 is associated with the presence of raised gammaglobulins.

On the whole these results are in agreement with previous data obtained in ethnically different individuals. In 1979 Moutsopoulos *et al* reported the association between DR3 and primary SS.⁵ In subsequent studies anti-Ro/La antibodies were found to be associated with DR2/DR3 in both primary and secondary SS,⁶ and in systemic lupus erythematosus.^{7,8} In addition, DR3 was the genetic marker more closely linked to the presence of anti-Ro antibodies in primary SS.⁶ On the other hand, DRw52 alloantigen was also found to be associated with both primary and secondary SS, although in these studies the relation between this specificity and extraglandular involvement of this disorder was not investigated.^{5,6}

Although this study confirms the strong genetic background of primary SS, the complex interplay between genetic markers and signs and symptoms of this disorder is not yet clearly defined. Undoubtedly the presence of DR3 seems to identify a particular subset of patients with primary SS, i.e., those with extraglandular manifestations and with peculiar marker antibodies, such as anti-Ro/La antibodies. Whether anti-Ro/La autoantibodies are of pathogenetic importance or represent simple epiphenomena favoured by the genetic background is also not yet clear. It is known that DR3 is closely associated with a number of immunological disorders¹⁸ and it is commonly thought to mediate the immune responsiveness to unknown extrinsic stimuli.⁸ On the other hand, some observations

suggest a direct pathogenetic role for these autoantibodies. For instance, Ro/anti-Ro immune complexes were eluted from the renal tissue of patients with lupus nephritis.¹⁹ Furthermore, transplacental passage of this antibody is commonly regarded to be responsible for the congenital heart block observed in children born from mothers with a high titre of anti-Ro antibodies.²⁰

Finally, a number of considerations suggests that the role of DRw52 in SS is subordinate to that of DR3. Although the genetic significance of DRw52 is still controversial,²¹ recent evidence indicates that this alloantigen is a 'semipublic' specificity located on certain DR molecules,²² and this may explain the association with DR3, DR5, and DR6 in this and other studies.^{6,8}

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