Penicillamine-induced myasthenia in rheumatoid arthritis: its clinical and genetic features

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SUMMARY The clinical features and genetic background of 18 patients with rheumatoid arthritis were investigated following the development of penicillamine-induced myasthenia (PIM). The initial myasthenic symptoms in all patients consisted of variable diplopia and/or ptosis with progression to a more generalised involvement in 7 of them. No clinical, humoral, or genetic factor was determined which would allow identification of individuals developing generalised as opposed to ocular myasthenia. Withdrawal of penicillamine was associated over 4–60 weeks with a slow resolution of symptoms, facilitated in 12 patients by the use of anticholinesterase agents. In 2 patients a persistent partial unilateral ptosis remains after 15 and 25 months, while in a further patient diplopia is present 42 months after resolution of the other myasthenic symptoms. The patients with PIM when compared with a healthy 'control' population had a significant increase in HLA Dr1 (p corr < 0.005) and an absence of HLA Dr 3. A genetic susceptibility to the development of PIM, distinct from that observed in myasthenia gravis of spontaneous onset, is suggested by this abnormal distribution of HLA Dr antigens.

Myasthenia is a recognised, albeit uncommon, complication of penicillamine therapy.¹ The majority of cases of this supposedly immune-mediated side effect have arisen when penicillamine is employed in the treatment of rheumatoid arthritis.¹ However, isolated cases have occurred in association with the treatment of Wilson's disease,^{2,3} progressive systemic sclerosis,⁴ and primary biliary cirrhosis.⁵ Penicillamine-induced myasthenia (PIM), while being dependent on penicillamine for its development and progression,¹ closely resembles spontaneous-onset myasthenia gravis clinically and in the accompanying presence of antibodies directed against acetylcholine receptors and/or striated muscle.⁶

The role of constitutional factors in the aetiology of spontaneous-onset myasthenia gravis and the development of immune-mediated manifestations of drug toxicity has recently aroused great interest. A genetic predisposition to the development of hydralazine-induced systemic lupus erythematosus,⁷ parenteral gold-induced nephropathy,⁸ and spontaneous-onset myasthenia gravis when as-

sociated with thymic hyperplasia^{9–11} has been inferred from the abnormal distribution of HLA antigens observed in these 3 pathological states.

We have investigated the possibility that a genetic factor, determined by a locus or loci situated within the major histocompatibility complex (MHC) of chromosome 6, might influence the development and/or expression of PIM. The HLA A, B, and Dr type together with the alleles of the complement components C2, C4, and factor B (which are controlled by loci in close proximity to those of HLA B and Dr) were ascertained in patients with rheumatoid arthritis who had developed a penicillamine-dependent myasthenia.

Patients and methods

Eighteen previously unreported Caucasian patients (4M:14F) who developed PIM during the period August 1978 to December 1981 were studied. All suffered with classical or definite rheumatoid arthritis as defined by the American Rheumatism Association criteria. Patient data together with details of penicillamine therapy and duration of rheumatoid arthritis at the onset of myasthenic symptoms appear in Table 1.

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Table 1 Patient data. Penicillamine therapy and duration of rheumatoid arthritis (RA) at the onset of myasthenia.

Subject	Age (yr)	Sex	Duration of RA (yr)	Penicillamine therapy	
				Duration (months)	Dosage (mg)
1	54	F	5	11	250
2	55	F	10	12	500
3	64	F	10	21	500
4	46	F	7	3	250
5	36	M	4	7	750
6	56	F	18	27	250
7	46	F	4	8	750
8	39	F	3	32	250
9	42	M	4	22	375
10	67	F	7	8	250
11	64	F	3	22	250
12	54	F	34	5	500
13	57	F	7	54	625
14	61	M	10	24	500
15	47	F	7	6	500
16	53	M	3	9	1500
17	53	F	12	24	1000
18	53	F	4	12	250

Myasthenia. The clinical diagnosis of myasthenia was substantiated by a rapid transient improvement in the myasthenic symptoms and signs following the intravenous administration of edrophonium chloride. Chest radiography was performed in an attempt to exclude the presence of a thymoma. Anti-acetylcholine receptor antibodies were determined by the iodinated α -bungarotoxin assay,¹² and rat striatal muscle was employed as substrate for the detection of anti-striated-muscle antibodies by indirect immunofluorescence.

HLA and complement typing. The HLA antigens at the A, B, and Dr loci were determined by the standard 7th Histocompatibility Workshop microcytotoxicity technique.¹³ Serum, which had been stored at -70°C and thawed immediately prior to analysis, was used to determine the alleles of the complement components C4¹⁴ and factor B (Bf)¹⁵ by the method of agarose electrophoresis followed by immunofixation. The alleles of complement C2 were obtained by isoelectric focusing as described by Meo *et al.*¹⁶

Statistical methods. Significance was determined by

Table 2 Initial and subsequent myasthenic symptoms and signs together with their duration.

Subject	Myasthenic symptoms and signs		Period of resolution
	Initial	Subsequent	
1	Diplopia and ptosis	None	17 Months, incomplete (persisting partial (L) ptosis)
2	Diplopia and ptosis	None	2 Months, complete
3	Diplopia	Ptosis, slurred speech, neck and shoulder girdle weakness	4 Months, complete
4	Ptosis	Generalised weakness, slurred speech	2 Months, complete
5	Diplopia	Ptosis generalised weakness, dyspnoea	2 Months, complete
6	Diplopia and ptosis	generalised weakness	4 Months, complete
7	Diplopia and ptosis	None	1 Month, complete
8	Ptosis	Shoulder girdle weakness, slurred speech	6 Months, complete
9	Diplopia	Ptosis, slurred speech	3 Months, complete
10	Ptosis	None	24 Months, incomplete (persisting partial (R) ptosis)
11	Diplopia	Ptosis, weakness jaw and neck. Dyspnoea	15 Months, complete
12	Diplopia and ptosis	None	Awaited
13	Diplopia and ptosis	None	Awaited
14	Diplopia and ptosis	None	Awaited
15	Diplopia and ptosis	Cervical and shoulder girdle weakness	Awaited
16	Diplopia and ptosis	None	8 Months, complete
17	Diplopia	Slurred speech	18 Months, incomplete (persisting diplopia)
18	Diplopia and ptosis	None	5 Months, complete

the chi-square test with Yates's correction. The corrected probability (p corr) was obtained by multiplying the uncorrected probability (p) by the number of antigens sought at that particular HLA locus (i.e. in the case of HLA Dr p corr = $p \times 9$).

Results

CLINICAL FEATURES

The initial and subsequent myasthenic symptoms and signs, which were not accompanied by any radiographic evidence of a thymoma, are illustrated in Table 2. Owing to delays in both presentation and diagnosis penicillamine therapy was continued after the initial onset of PIM for a period of 1–96 weeks (mean 12 weeks). During this time progression to more generalised myasthenia occurred in 7 patients, 2 of whom (patients 5 and 11) had involvement of the muscles of respiration. The initial myasthenic symptoms and signs in the remaining 11 patients persisted essentially unchanged, although they continued to receive penicillamine for 2–96 weeks (mean 16 weeks). In 2 patients (nos. 12 and 14), whose presenting features of diplopia and ptosis had persisted, but not progressed, for 1 and 3 months respectively the dosage of penicillamine was decreased but not discontinued as in the other 16 patients, 12 of whom required treatment with anticholinesterase agents. In the 14 patients who were studied for 4 months or more after presentation complete and permanent resolution of all myasthenic features occurred in 11 (Table 2). In the remaining 3 patients (nos. 1, 10 and 17) a persistent partial ptosis remained in 2 subjects (nos. 1 and 10) and diplopia in the third. Subjectively, penicillamine therapy was associated in all patients with a marked improvement in rheumatoid disease activity, which remained unaltered at the onset of myasthenia. Concomitant with this onset one patient (no. 8) developed a systemic lupus erythematosus-like syndrome with an antinuclear factor (ANF) titre 1/2560 and anti-DNA antibodies of 55 units/ml (normal range <25 units/ml), while a further patient (no. 2) developed histologically confirmed hepatotoxicity. Both these toxic side effects, along with PIM, resolved spontaneously on withdrawal of penicillamine. In the 17 subjects tested, antibodies directed against acetylcholine receptors and striatal muscle were present in 17 and 4 respectively.

HLA AND COMPLEMENT TYPING

The frequency of the HLA B and Dr antigens in both the patients studied and in two 'local' control populations are illustrated in Tables 3 and 4. Minor differences in the distribution of the HLA B antigens, in particular HLA B27, were detected in the PIM

Table 3 Incidence of HLA B antigens in study group and Birmingham control population

HLA B antigen	Study series (18 subjects)	Control population (380 subjects)
B5	1 (6%)	36 (9%)
B7	4 (22%)	125 (33%)
B8	5 (28%)	114 (30%)
B12	8 (44%)	132 (35%)
B13	1 (6%)	11 (3%)
B14	0 (0%)	20 (5%)
B15	1 (6%)	41 (11%)
B16	2 (11%)	20 (5%)
B17	1 (6%)	32 (8%)
B 18	1 (6%)	20 (5%)
Bw21	1 (6%)	9 (2%)
Bw22	0 (0%)	11 (3%)
B27	5 (28%)	32 (8%)
Bw35	2 (11%)	40 (10%)
B37	0 (0%)	6 (1%)
B40	1 (6%)	40 (10%)

Table 4 Incidence of HLA Dr antigens in study group and Birmingham control population.

HLA Dr antigen	Study series (16 subjects)	Control population (59 subjects)
Dr 1	10 (62%)*	10 (17%)
Dr 2	3 (19%)	18 (30%)
Dr 3	0	18 (30%)
Dr 4	5 (31%)	13 (22%)
Dr 5	0	6 (10%)
Dr w 6	2 (12%)	8 (13%)
Dr 7	6 (37%)	15 (25%)
Dr w 8	0	1 (2%)
Dr w 9	1 (6%)	5 (8%)

*HLA DR 1 significantly increased in study population (p corr <0.005).

patients compared with the control group. However, none of these was statistically significant. It proved technically impossible in subjects 4 and 9 to obtain a satisfactory HLA Dr type. In the remaining 16 patients there was a significant increase in HLA Dr 1 (62% PIM; 17% controls; p corr <0.005) and an absence of HLA Dr 3. Complement typing in the 16 subjects investigated showed 13 to be homozygous for the electrophoretically 'slow' allele of complement factor B (BfSS), 2 patients (no. 3 and 7), both of whom were HLA B8, were homozygous for the 'fast' allele (BfFF), and patient 6 (HLA B7B18) was heterozygous (BfFS). No abnormal distribution of the alleles of the complement components C2 and C4 was found.

Discussion

The aetiology of this distressing and occasionally fatal manifestation of penicillamine toxicity remains obscure. However, the suggestion that penicillamine might in some undetermined manner unveil latent myasthenia gravis has been the subject of debate.¹⁷⁻²⁰ The data arising from our study do not support this suggestion. HLA typing in the PIM subjects revealed a significant increase in HLA Dr 1 and an absence of HLA Dr 3. These findings argue that subjects developing PIM possess a different genetic background from those suffering spontaneous-onset myasthenia (in the absence of a thymoma), where the incidence of HLA Dr 3 is increased and that of HLA Dr 1 is normal.⁹⁻¹¹ They also appear to differ genetically from Caucasian rheumatoid patients in general, in whom HLA Dr 4 is increased but the incidence of HLA Dr 1 is unaltered.²¹⁻²³

The literature contains reports of 6 other cases of PIM complicating rheumatoid arthritis in which HLA A, B, and Dr typing has been performed.^{6 20 24 25} Four cases were HLA Dr 1, while one was HLA Dr 3.²⁰ The myasthenia in this last case was, however, atypical in the absence of antiacetylcholine receptor antibodies and, more importantly, in the recurrence of myasthenia 6 months after its initial complete resolution and 8 months after penicillamine had been withdrawn, a course that makes a drug-induced aetiology unlikely. The absence of HLA Dr 3 in our study might reflect the increase in HLA Dr 1 which was observed in 62% of our patients as compared with 17% of controls. An alternative explanation for this apparent loss of the normal linkage disequilibrium between HLA B8 and Dr 3 would be the conferment of resistance to the development of PIM by the HLA B8 Dr 3 haplotype.

Two of our 5 HLA B8 subjects were B8FF, as opposed to none of 100 normal HLA B8 subjects investigated by Arnason *et al.*²⁶ This confirms either that there has been a recombination of the known allelic association, or that we have detected an unusual MHC haplotype.

The initial myasthenic symptoms and signs observed in this study together with their progression and association with other manifestations of penicillamine toxicity are similar to the findings reported by others.¹⁷ Unfortunately we detected no clinical, serological, or genetic marker which would allow early differentiation between the group of 7 patients who developed generalised symptoms and the 11 whose initial myasthenic features remained essentially unchanged, even though penicillamine therapy was continued in some cases for several months. Owing to this inability to prognosticate between progressive and nonprogressive cases, discontinuation of

penicillamine at the onset of symptoms, with or without the use of anticholinesterase agents, is the recommended regimen. Penicillamine usage was nevertheless associated with a striking improvement in rheumatoid disease activity. In view of this and the late presentation of the majority of patients with solely ocular symptoms, a closely monitored trial of decreasing dosage, rather than discontinuance of penicillamine, has been instituted in 2 patients with nonprogressive ocular myasthenia. It is too early to comment on the results of this modified therapy.

Although a genetic susceptibility to the development of PIM, based on the increased incidence of HLA Dr 1 and/or absence of HLA Dr 3, requires to be substantiated, these results strongly suggest that a different genetic background characterises drug-induced as opposed to spontaneous-onset myasthenia. It is worth noting that an absence of HLA Dr 3 has been observed in another example of drug-induced autoimmunity. Batchelor *et al.*⁷ found none of 26 patients with hydralazine-induced systemic lupus erythematosus to be HLA Dr 3 positive. In a preliminary report on rheumatoid patients who developed gold- or penicillamine-induced thrombocytopenia Panayi *et al.*²⁷ found the HLA Dr 3 antigen to be present in all 7 patients who developed a rapid fall in platelets but absent in 10 who suffered a more gradual decline in the platelet count. But the mechanisms underlying the thrombocytopenia were not defined. The fascinating possibility that the HLA B8 Dr 3 haplotype might predispose to spontaneous-onset autoimmune disorders while offering resistance to their drug-induced counterparts warrants further investigation.

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