Discussion

Scleroderma of the lung¹ and the proximal muscles² has been well described but not, to our knowledge, scleroderma of the diaphragm. Diaphragmatic symptoms have been described in systemic lupus erythematosus,³ but the clinical picture and low level of anti-DNA antibody and the findings at necropsy militate against a diagnosis of this or of mixed connective tissue disease. The raised activity of creatinine phosphokinase in this patient suggest that the myopathy may have fitted into the pattern of inflammatory myopathy,⁴ which might, therefore, have responded to treatment with corticosteroids. We suggest that the extensive fibrosis with accompanying degeneration of the myofibrils within skeletal type muscle in the diaphragm (figure) was secondary to scleroderma and a contributing factor to the terminal respiratory failure. Diaphragmatic changes should, therefore, be added to the list of respiratory complications in scleroderma.

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References

- ¹ Bader RA, Bader ME. Thoracic manifestations of collagen diseases. In: Baum GL, ed. *Textbook of pulmonary disease*. 2nd ed. Boston: Little, Brown and Co, 1974:463-7.
- ² Rodnan GP. Progressive systemic sclerosis (scleroderma). In: Hollander JL, McCarty DJ Jr, eds. *Arthritis and allied conditions*. Philadelphia: Lea and Febiger, 1972:962-1005.
- ³ Gibson GJ, Edmonds JP, Hughes GRV. Diaphragm function and lung involvement in systemic lupus erythematosus. *Am J Med* 1977;63:926-32.
- ⁴ Clements PJ, Furst DE, Campion DS, et al. Muscle disease in progressive systemic sclerosis. *Arthritis Rheum* 1978;21:62-71.

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HLA antigens and acetylcholine receptor antibodies in penicillamine induced myasthenia gravis

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Abstract

Antibodies to the acetylcholine receptor and HLA antigens have been studied in patients with myasthenia gravis occurring in association with penicillamine treatment. The properties of the antiacetylcholine receptor in these patients differed from those in patients with idiopathic myasthenia gravis in terms of specificity and affinity. These patients had an increased prevalence of HLA Bw35 and DR1 compared to controls and a decreased frequency of B8 and DR3 compared to patients with idiopathic myasthenia gravis. Likewise, they had a decreased frequency of DR4 compared to patients with rheumatoid arthritis.

These data provide supportive evidence for a role for penicillamine in the induction of myasthenia gravis in genetically predisposed individuals.

Introduction

For many years penicillamine has been used in the treatment of rheumatoid arthritis. The drug has been implicated in the induction of several autoimmune diseases, including myasthenia gravis.¹ The myasthenia gravis induced by penicillamine is clinically indistinguishable from idiopathic myasthenia gravis and is invariably associated with the presence of antibodies to the acetylcholine receptor. After withdrawal of penicillamine, titres of antiacetylcholine receptor usually fall and myasthenia gravis resolves.¹

The aetiological agent in idiopathic myasthenia gravis is unknown. In Caucasians the HLA antigens B8 and DR3 are known to be increased, particularly in those patients with early onset of disease and thymic hyperplasia.²⁻³ By contrast there is

an increase in the prevalence of DR4 in patients with rheumatoid arthritis.⁴

We have previously reported that antiacetylcholine receptor in patients with penicillamine induced myasthenia gravis is less cross reactive than that from patients with idiopathic myasthenia gravis.⁵ Antiacetylcholine receptor in penicillamine induced myasthenia gravis tends to have a higher average affinity (see below). These data suggest that penicillamine induced myasthenia gravis is subtly different from the idiopathic disease and support the conclusion that penicillamine may induce myasthenia gravis.

The identification of genetic factors that predispose to the induction of myasthenia gravis by penicillamine would provide further such evidence. More importantly, the identification of a susceptible genetic background, combined with the knowledge of the aetiological agent, would provide a unique opportunity to study the pathogenesis of myasthenia gravis.

In a preliminary report we noted that Caucasian patients with penicillamine induced myasthenia gravis have a higher than expected prevalence of Bw35 and DR1.⁶ We now present the full typing data and compare prevalences of HLA types in these patients with those in patients with rheumatoid arthritis, patients with idiopathic myasthenia gravis, and controls.

Patients and methods

Clinical details and sera were supplied by several clinicians throughout Australia, New Zealand, United Kingdom, and the United States of America. All but two patients were being treated for rheumatoid arthritis. One of the exceptions was receiving penicillamine for scleroderma⁷ and the other for Wilson's disease.⁸ All were Caucasian.

Tissue typing was performed using standard microlymphocytotoxicity techniques.⁹ Typing was done in the patients' own area and the HLA types supplied by their clinicians. Details on 12 patients were collected by this laboratory. These have been combined with details of four cases whose HLA data and antiacetylcholine receptor titre have been published by others.^{7,10-12} All 16 patients were typed for HLA-A and HLA-B antigens and 10 of these were also typed for DR.

Since the patients were derived from several centres throughout the world an appropriate control group was difficult to obtain. Antigen prevalences have been compared with those found in healthy Cauca-

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sians in the Second Asia Oceania Histocompatibility Workshop Conference and Caucasian patients with rheumatoid arthritis who were typed for HLA antigens in this laboratory for the Second Asia Oceania Histocompatibility Workshop Conference. Statistical comparisons were made using the binomial test taking the prevalences of phenotypes in the comparison groups as the probabilities of occurrence of particular antigens.

Antiacetylcholine receptor was measured in 43 patients with penicillamine induced myasthenia gravis by previously published methods and titres were expressed in arbitrary units.¹³ Antiacetylcholine receptor affinity was determined by the method of Odell *et al.*¹⁴ A standard volume of serum (5 μ l) was reacted against increasing concentrations of acetylcholine receptor and precipitated with goat antihuman IgG.¹³ IgG present in the crude preparations of acetylcholine receptor was routinely removed by passage through Sepharose anti-IgG columns (Garlepp *et al.*, unpublished observations). In excess of acetylcholine receptor a plateau of binding was reached and the average affinity of the antiacetylcholine receptor was determined from the reciprocal of the free concentration of acetylcholine receptor at 50% saturation (fig 1). This technique was preferred to Scatchard analysis, which is best applied to systems in which there is non-cooperative binding of univalent ligands of homogeneous affinity to their receptors.¹⁴

FIG 1—Determination of binding affinity of antiacetylcholine receptor (AChR). Points a and b represent 50% antibody saturation for serum samples 1 and 2 respectively: free concentration at these points may be calculated, by subtraction, from the AChR added.

Results

ANTIACETYLCHOLINE RECEPTOR

Figure 2 shows titres of antiacetylcholine receptor in patients with penicillamine induced myasthenia gravis. All four patients who had titres <1 unit were in remission at the time of assay.

Table I shows the average binding affinities and titres of antiacetylcholine receptor in patients with idiopathic (n=14) and penicillamine induced (n=11) myasthenia gravis. Patients with penicillamine induced disease had higher affinities (p=0.025; Mann-Whitney U test).

HLA TYPES

Table II shows the HLA types of 16 patients with penicillamine induced myasthenia gravis. Table III shows the prevalences of selected antigens in these patients compared with their prevalences in patients with idiopathic myasthenia or rheumatoid arthritis and those in Caucasian controls.

Prevalences of Bw35 and DR1 and of the combination Bw35 and DR1 were increased in patients with penicillamine induced myasthenia gravis compared to patients in all other groups. By contrast prevalences of B8 and DR3 and the combination A1, B8, DR3 were increased in patients with idiopathic myasthenia gravis and DR4 was increased in patients with rheumatoid arthritis.

Discussion

These results show that although penicillamine induced and idiopathic myasthenia gravis are both associated with antiacetylcholine receptor and are clinically identical they do differ in some respects.

We have previously reported that antiacetylcholine receptor from patients with penicillamine induced disease show only

FIG 2—Titres of antiacetylcholine receptor (AChR) in 43 patients with penicillamine-induced myasthenia gravis. ○=Patient in remission.

TABLE I—Affinities of antiacetylcholine receptor in serum from patients with myasthenia gravis

Idiopathic			Penicillamine induced		
Case No	Titre (units)	Affinity (l/mol $\times 10^{10}$)	Case No	Titre (units)	Affinity (l/mol $\times 10^{10}$)
1	80	7.8 $\pm$ 0.3*	15	2	11.6 $\pm$ 0.9*
2	22	5.9 $\pm$ 0.5	16	25	9.9 $\pm$ 1.1
3	180	5.7 $\pm$ 0.8	17	9	8.6 $\pm$ 0.9
4	25	5.3 $\pm$ 1.0	18	9	8.3
5	2100	5.1	19	36	8.0 $\pm$ 1.2
6	12	4.5 $\pm$ 0.5	20	17	7.0
7	47	4.3 $\pm$ 0.8	21	13	5.5
8	250	4.1 $\pm$ 1.0	22	8	4.8
9	12	3.2 $\pm$ 0.1	23	9	2.9 $\pm$ 0.4
10	25	2.6	24	15	2.7
11	43	2.3	25	5	<0.9
12	100	2.1			
13	25	<1.3			
14	22	<0.7			
Mean		3.9 $\pm$ 1.9			6.4 $\pm$ 3.2

* Mean of 2-5 experiments where SD is shown.

TABLE II—Clinical details of 16 patients with penicillamine induced myasthenia gravis*

Case No	Age	Sex	Anti-AChR	HLA type		
				A	B	DR
15	52	F	+	2, 3	7, 27	2, w6
17	39	F	+	1, 2	8, 18	2, 7
20	65	F	+	1, 2	w35, 40	1, w6
21	24	F	+	11, w24	27, w35	NA
22	64	F	+	3, 11	7	1
23	NA	F	+	1, 11	8, w35	1, 3
24	21	F	+	3, 11	14, 37	NA
26	42	F	+	3, w24	w35	NA
27	70	F	+	2, 23	18, w35	1, w6
28	NA	F	+	1, 3	7, w49	1, 4
29	NA	M	+	1, 3	w35, w57	1, 7
30	NA	F	+	2, 3	7, 8	NA
31	NA	F	NA	w31, w32	w35, w39	5, w8
32	48	F	+	1	27, w44	NA
33	34	F	+	3, 11	5, 8	NA
34	52	F	+	3, 11	w35	1, 8

AChR = Acetylcholine receptor. NA = Not available.

* Cases 21, 24, 32, 33, and 34 were reported in references 7, 8, 11, 10, and 12 respectively.

TABLE III—Prevalence of HLA antigens in patients with penicillamine induced myasthenia gravis compared to that in patients with idiopathic myasthenia gravis or rheumatoid arthritis and in controls. Figures are numbers (%) of patients

	Drug induced myasthenia gravis (n = 16)	Rheumatoid arthritis (n = 52)	Idiopathic myasthenia gravis (n = 35)	Controls* (n = 108)
<i>A and B types</i>				
A1	7 (44)	20 (38)	22 (64)	45 (42)
A11	5 (31)	7 (13)	2 (6)	8 (7)
B8	4 (25)	18 (36)	19 (54)	30 (28)
Bw35	8 (50)†	8 (15)†	5 (14)†	12 (11)†
<i>DR types‡</i>				
DR1	7 (70)§	9 (20)§	7 (20)§	19 (18)§
DR3	1 (10)‖	10 (23)	16 (46)	25 (23)
DR4	1 (10)†	30 (67)†	7 (20)	35 (32)
Bw35/DR1	5 (50)	2 (5)	1 (3)	5 (5)
A1/B8/DR3	1 (10)	5 (11)	16 (46)	19 (18)

* Controls taken from cases reported at Second Asia Oceania Histocompatibility Workshop.

† p < 0.005.

‡ DR typing was undertaken in only 10 of the 16 patients with penicillamine induced myasthenia gravis and in 44 of the 52 with rheumatoid arthritis.

§ p < 0.001.

‖ p < 0.05.

limited cross reactivity with xenogeneic acetylcholine receptor.⁵ This lack of cross reactivity is not merely due to inadequate test concentrations of acetylcholine receptor or to short incubation times as has been suggested.¹⁵ Using Scatchard analysis Vincent and Newsom-Davis¹⁵ have reported that penicillamine induced antiacetylcholine receptor had lower avidity than antiacetylcholine receptor from patients with longstanding idiopathic myasthenia gravis. The inherent limitations mentioned above usually result in curved Scatchard plots when antibody affinity is measured in this way. It is also necessary to remove, or allow for, contaminating IgG from the preparation of acetylcholine receptor if increasing concentrations are to be used (Garlepp *et al*, unpublished observations). Avidity was said to increase with time of incubation.¹⁵ Bray and Drachman,¹⁶ also using Scatchard analysis, but using sera at high dilution and having effectively removed IgG from their preparations of acetylcholine receptor, have reported average affinities of antiacetylcholine receptor in idiopathic myasthenia gravis similar to those reported here. Our data show that antiacetylcholine receptor in patients with penicillamine induced myasthenia gravis was generally of higher average affinity than that from patients with idiopathic disease.

The HLA antigens Bw35 and DR1 were associated with penicillamine induced myasthenia gravis. The increased prevalence of DR1 in patients with penicillamine induced disease has recently been confirmed.¹⁷ The relative absence of DR4 from the patients with penicillamine induced disease also suggests that these patients are genetically distinct from the remaining patients with rheumatoid arthritis. DR1 has, however, been shown to be increased in patients with rheumatoid arthritis in two different racial groups^{18, 19} while Bw35 and DR1 may be associated with DR4-negative rheumatoid arthritis in Caucasians (P Kay *et al*, unpublished observations).

The relative scarcity of B8 and DR3 in patients with penicillamine induced myasthenia gravis (see also reference 17) contrasts with its increased prevalence in patients with idiopathic myasthenia gravis, particularly in women with early onset. Interestingly Bw35 and DR1 do influence susceptibility to myasthenia gravis especially in Asian Indians.²⁰ Thus one interpretation of our data would be that DR3 and DR4 favour myasthenia gravis and rheumatoid arthritis respectively, whereas the combination of Bw35 and DR1 permits both. Alternatively Bw35 and DR1 may allow a specific reaction with penicillamine or may be a marker for genetic factors affecting immunoregulation.²¹

Some interesting parallels exist. Insulin dependent diabetes mellitus has been shown to be associated with more than one HLA supertype.²² Hydralazine induced systemic lupus erythematosus is associated with DR4 although the idiopathic disease is not.²³

The temporal relationship between administration or withdrawal of penicillamine and onset or remission of disease, the subtle differences in properties of antiacetylcholine receptor, and the distinct HLA association all indicate that penicillamine may induce myasthenia gravis. The demonstration of the association with HLA-Bw35 and DR1 should allow progress in investigation of the mechanisms of induction of antiacetylcholine receptor and myasthenia gravis.

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References

- Dawkins RL, Garlepp M, McDonald B, Williamson J, Zilko PJ, Carrano J. Myasthenia gravis and D-Penicillamine. *J Rheumatol* 1981;8,suppl 7: 169-72.
- Pirskanen R. On the significance of HL-A and LD antigens in myasthenia gravis. *Ann NY Acad Sci* 1976;274:451-60.
- Dawkins RL. Myasthenia gravis. In: Terasaki PI, ed. *Histocompatibility testing 1980*. Los Angeles: UCLA Tissue Typing Laboratory, 1980: 662-7.
- Stastny P. Rheumatoid arthritis. In: Terasaki PI, ed. *Histocompatibility testing 1980*. Los Angeles: UCLA Tissue Typing Laboratory, 1980: 681-6.
- Garlepp MJ, Kay P, Dawkins RL, Bucknall R, Kemp A. Cross-reactivity of anti acetylcholine receptor autoantibodies. *Muscle Nerve* 1981;4: 282-6.
- Garlepp MJ, Christiansen FT, Dawkins RL. Association of HLA Bw35, DR1 with D-Penicillamine induced myasthenia gravis. *Aust NZ J Med* 1982;12:559.
- Torres CF, Griggs RC, Baum J, Penn AS. Penicillamine-induced myasthenia gravis in progressive systemic sclerosis. *Arthritis Rheum* 1980;23:505-8.
- Masters CL, Dawkins RL, Zilko PJ, Simpson JA, Leedman RJ, Lindstrom J. Penicillamine-associated myasthenia gravis: anti acetylcholine receptor and antistriational antibodies. *Am J Med* 1977;63:689-94.
- Terasaki PI, ed. *Histocompatibility testing 1980*. Los Angeles: UCLA Tissue Typing Laboratory, 1980.
- Russell AS, Lindstrom J. Penicillamine-induced myasthenia gravis associated with antibodies to the acetylcholine receptor. *Neurology* 1978;28:847-9.
- Albers JW, Hodach RJ, Kimmel DW, Treacey WL. Penicillamine-associated myasthenia gravis. *Neurology* 1980;30:1246-50.
- Keesey J, Novom S. HLA antigens in penicillamine-induced myasthenia gravis. *Neurology* 1979;29:528-9.
- Garlepp MJ, Kay P, Dawkins RL. The diagnostic significance of autoantibodies to the acetylcholine receptor. *Journal of Neuroimmunology* (in press).
- Odell WD, Abraham GA, Skowsky WR, Hescoc MA, Fisher DA. Production of antisera for radioimmunoassays. In: Odell WD, Daughaday WH, eds. *Principles of competitive binding assays*. Philadelphia: Lippincott Co, 1971:57-76.
- Vincent A, Newsom-Davis J. Acetylcholine receptor antibody characteristics in myasthenia gravis. II. Patients with penicillamine-induced myasthenia or idiopathic myasthenia of recent onset. *Clin Exp Immunol* 1982;49:266-72.
- Bray JJ, Drachman DB. Binding affinities of anti acetylcholine receptor autoantibodies in myasthenia gravis. *J Immunol* 1982;128:105-10.
- Delamere JP, Jobson S, Mackintosh LP, Wells L, Walton KW. Penicillamine-induced myasthenia in rheumatoid arthritis: its clinical and genetic features. *Ann Rheum Dis* (in press).
- Woodrow JC, Nichol FE, Zaphiropoulos G. DR antigens and rheumatoid arthritis: a study of two populations. *Br Med J* 1981;283:1287-8.
- Schiff B, Mizrahi Y, Orgad S, Yaron M, Gazit E. Association of HLA-AW31 and HLA-DR1 with adult rheumatoid arthritis. *Ann Rheum Dis* 1982;41:403-4.
- Mehra NK, Ahuja GK, Vaidya MC. HLA antigen frequencies in myasthenia gravis in North India. In: Dawkins RL, Christiansen FT, Zilko PJ, eds. *Immunogenetics in rheumatology*. Amsterdam: Excerpta Medica 1982:264-6.
- Zilko PJ, Dawkins RL, Holmes K, Witt C. Genetic control of suppressor lymphocyte function in myasthenia gravis: relationship of impaired suppressor function to HLA B8/DR3 and cold reactive lymphocytotoxic antibodies. *Clin Immunol Immunopathol* 1979;14:222-30.
- Dawkins RL, Christiansen FT, Kay PH, *et al*. Disease associations with campotypes, supratypes and haplotypes. *Immunol Rev* 1982;70:5-22.
- Batchelor JR, Welsh KI, Tinoco RM, *et al*. Hydralazine-induced systemic lupus erythematosus: influence of HLA-DR and sex on susceptibility. *Lancet* 1980;i:1107-9.

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