CONCISE REPORTS

Gold induced nephropathy in rheumatoid arthritis and HLA class II genes

L I Sakkas, I C Chikanza, R W Vaughan, K I Welsh, G S Panayi

Abstract

Objectives—To elucidate the role of HLA-DRB, -DQA, and -DQB genes in patients with rheumatoid arthritis (RA) who developed gold induced nephropathy.

Methods—Southern blot analysis of HLA-DRB, -DQA, and -DQB genes was performed on DNA from 27 patients with RA with gold induced nephropathy, 37 patients with RA who were treated with gold but did not develop nephropathy, and 122 ethnically matched normal subjects.

Results—The 4.7 kb DQA1/Taq I band associated with DQA1*0501 and DR3 and DR5 was found in 16 (59%) patients with gold induced nephropathy compared with five (14%) patients without gold induced nephropathy.

Conclusion—It is concluded that HLA-DQA region genes may be an important susceptibility factor for the development of gold induced nephropathy in patients with RA.

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Nephropathy is a common side effect of sodium aurothiomalate treatment in patients with rheumatoid arthritis (RA) and is manifested as proteinuria. Serological HLA-DR studies have shown that gold induced nephropathy is associated with the HLA-DR3 antigen,¹⁻⁴ but this is not a universal finding.^{5,6} A few workers have suggested that the HLA-DR3 haplotype rather than DR3 itself may be important for the development of this complication.⁷ In this study we used restriction fragment length polymorphism (RFLP) to analyse the HLA-DRB, -DQA, and -DQB genes in patients with RA with gold induced nephropathy.

Patients and methods

Twenty seven patients with RA (12 men) who developed proteinuria (>0.5 g/24 hours) after treatment with sodium aurothiomalate injections and who did not have urinary tract infections were studied. In these patients proteinuria cleared after the gold treatment was discontinued. Twenty one patients were seropositive. As controls we used 37 patients with RA who were treated with the same preparation of gold for at least six months but who did not develop proteinuria. The two patient groups were white English subjects with classical or definite RA (American Rheumatism Association criteria) attending the rheumatology clinics at Guy's and Lewisham Hospitals. One hundred and twenty two ethnically matched normal subjects were also included in the study as a further control group. DNA extraction from peripheral blood, Southern blotting, and hybridisation with HLA-DRB, -DQA, and -DQB cDNA probes were carried out as described previously.⁸ Certain HLA-DR assignments were confirmed with the polymerase chain reaction (PCR) and sequence specific oligonucleotide probes.⁹

For statistical analysis the χ^2 test with Yates's correction and Fisher's exact test were used.

Results

Restriction fragment length polymorphisms associated with DR1-18 and DQw1-9 were identified according to Cox *et al*¹⁰ and Noreen *et al*.¹¹ HLA-DR3 specificities were divided into two subtypes which reflect a polymorphism of the DRB3 gene: DR3a characterised by the 11.6 kb DRB/Taq I band identifies the DRB3*0101 allele and is associated with HLA-B*0801, and DR3b characterised by the 13.6 kb DRB/Taq I band identifies the DRB3*02 allele and is associated with HLA-B*1801. All DR3 positive patients with gold induced nephropathy were DR3a, but neither DR3a nor DR3 frequencies were significantly increased compared with patients without gold induced nephropathy ($p=0.1$). Interestingly, the frequency of DR5 was significantly increased in the group with gold induced nephropathy compared with the group without gold induced nephropathy (Fisher's exact test; $p=0.0005$). The 4.7 kb DQA/Taq I band, associated with DQA1*0501, DR3, and DR5, was present in 59% of patients with gold

Molecular Immunogenetics, Tissue Typing and Rheumatology Units, Division of Medicine, United Medical and Dental Schools of Guy's and St Thomas's Hospitals, London SE1, United Kingdom

L I Sakkas
I C Chikanza
R W Vaughan
K I Welsh
G S Panayi

Correspondence to:
L I Sakkas,
Department of Medicine,
Medical School, University
of Ioannina,
451 10 Ioannina, Greece.

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Table 1 Distribution of selected HLA class II specificities in patients with rheumatoid arthritis treated with sodium aurothiomalate

HLA specificity	Patients with proteinuria (%)		p Value	Relative risk
	Present	Absent		
DR1	22	24		
DR3	33	14	0.1	3.2
DR4	44	68		
DR5	30	0	0.0005*	
4.7 kb DQA/Taq I band	59	14	0.0003	9.3

*Fisher's exact test.

Table 2 Polymerase chain reaction and sequence specific oligonucleotide probes used for the assignment of HLA-DR3 and -DR5(DRB1*11) specificities

	Codon	Strand	Sequence (5'-3')
5' Primer	15-21	Antisense	TGTCATTCTTCAATGGGACG
3' Primer	88-94	Sense	TCGCCGCTGCACTGTGAAG
DR3 Probe	74-80	Antisense	TGCAGTAGTTGTCCACC
DR5 Probe	55-61	Antisense	AGTACTCCTCATCAGGC

induced nephropathy and in 14% of patients without gold induced nephropathy (p=0.0003) (table 1). In normal subjects the frequencies of DR3, DR5, and 4.7 kb DQA/Taq I band were 36, 13, and 46% respectively. The assignment of HLA-DR3 and -DR5 (DRB1*11: seven patients with gold induced nephropathy) was confirmed using the PCR and sequence specific oligonucleotide probes (table 2).⁹

Discussion

Gold induced nephropathy is a particularly interesting entity as it represents an example of the interaction between environmental and genetic factors. In this light it could elucidate the pathogenesis of idiopathic membranous nephropathy. The latter disorder shares similar histological changes in the glomerulus with gold induced nephropathy. The mechanism by which sodium aurothiomalate causes nephropathy is not exactly known. After administration, sodium aurothiomalate dissociates into gold(I), which is transported to the tissues, and thiomalate. It is proposed that gold(I) is gradually oxidised into gold(III),¹² possibly in the phagolysosomes of macrophages.¹³ Gold(III) is a powerful protein thiol oxidiser and thus capable of creating new epitopes that can be seen by T cells. T cell sensitisation to gold(III) has been shown in animal studies.¹² This concept is consistent with the failure to detect gold in the subepithelial immune complexes in the glomerulus.¹⁴ It is also consistent with the HLA association of gold induced nephropathy, which may imply that T cells see antigen(s) in conjunction with HLA molecules.

This study has found a non-significant increase in the frequency of HLA-DR3 and a significant increase of DR5 and the 4.7 kb DQA1/Taq I band which is associated with the DQA1*0501 allele. Interestingly, it has been reported that this band is associated with

idiopathic membranous nephropathy.¹⁵ There is only one report of HLA-DQ typing and that has suggested that the DR3 association may be secondary to a further gene on the DR3 haplotype.⁷ It is, therefore, reasonable to speculate within the bounds of RFLP analysis that the DQA1 gene may be an important factor for the development of gold induced nephropathy.

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1 Wooley P H, Griffin J, Panayi G S, Batchelor J R, Welsh K I, Gibson T J. HLA-DR antigens and toxic reaction to sodium aurothiomalate and D-penicillamine in patients with rheumatoid arthritis. *N Engl J Med* 1980; 303: 300-2.

2 Gran T J, Husby G, Thorsby E. HLA antigens and gold toxicity. *Ann Rheum Dis* 1983; 42: 63-6.

3 Scherak O, Smolen J S, Mayr W R, Mayrhofer F, Kolarz G, Thumb N J. HLA antigens and toxicity to gold and penicillamine in rheumatoid arthritis. *J Rheumatol* 1984; 11: 610-4.

4 Hakala M, van Assendelft A H W, Ilonen J, Jalava S, Tiilikainen A. Association of different HLA antigens with various toxic effects of gold salts in rheumatoid arthritis. *Ann Rheum Dis* 1986; 45: 177-82.

5 Dequeker J, van Wange P, Verdict W. A systematic survey of HLA-A, B, C and D antigens and drug toxicity in rheumatoid arthritis. *J Rheumatol* 1984; 11: 282-6.

6 Alarcon G S, Barger B O, Acton R T, Koopman W J. HLA antigens and gold toxicity in American blacks with rheumatoid arthritis. *Rheumatol Int* 1986; 6: 13-7.

7 Singal D P, Reid B, Green D, D'Souza M, Bensen W G, Buchanan W W. Polymorphism of major histocompatibility complex extended haplotypes bearing HLA-DR3 in patients with rheumatoid arthritis and gold-induced thrombocytopenia or proteinuria. *Ann Rheum Dis* 1990; 49: 582-6.

8 Sakkas L I, Loqueman N, Bird H, Vaughan R W, Welsh K I, Panayi G S. HLA-class II and T cell receptor gene polymorphisms in psoriatic arthritis and psoriasis. *J Rheumatol* 1990; 17: 1487-90.

9 Vaughan R W, Lanchbury J S S, Marsh S G E, Hall M, Bodmer J G, Welsh K I. The application of oligonucleotide probes to HLA class II typing of the DRB subregion. *Tissue Antigens* 1990; 36: 149-55.

10 Cox N J, Mela A P, Zmijewski C M, Spielman R S. HLA-DR typing at the DNA level: RFLPs and subtypes detected with a DRB cDNA probe. *Am J Hum Gen* 1988; 43: 954-63.

11 Noreen H J, Davidson M L, McCullough J, Bach F H, Segal M. HLA class II typing by restriction fragment length polymorphism (RFLP) in unrelated bone marrow transplant patients. *Transplant Proc* 1989; 21: 2968-70.

12 Schuhmann D, Kubicka-Muranyi M, Mirtschewa J, Gunther J, Kind P, Gleichmann E. Adverse immune reactions to gold. I. Chronic treatment with Au(I) drug sensitizes mouse T cells not to Au(I) but to Au(III) and induces autoantibody formation. *J Immunol* 1990; 145: 2132-9.

13 Sharma R P, McQueen E G. The subcellular distribution of gold in monkey liver, kidney and spleen in vivo. *Clin Exp Pharmacol Physiol* 1979; 6: 561-3.

14 Strunk S W, Ziff M. Ultrastructural studies of the passage of gold aurothiomalate across the renal glomerular capillary wall. *Arthritis Rheum* 1970; 13: 39-72.

15 Vaughan R W, Demaine A G, Welsh K I. A DQA1 allele is strongly associated with idiopathic membranous nephropathy. *Tissue Antigens* 1989; 34: 261-9.