HLA ANTIGENS AND RESPONSE TO ANTIRHEUMATIC DRUGS

FETZE SPEERSTRA

HLA ANTIGENS AND RESPONSE TO ANTIRHEUMATIC DRUGS

PROEFSCHRIFT

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mijn ouders

The studies presented in this thesis were performed in the outpatient clinic of the Division of Rheumatology (Prof.Dr L.B.A. van de Putte) of the Sint Radboud Hospital, Nijmegen and in the Institute of Transplantation Serology, Blood Transfusion Service (Prof.Dr V. Kunst), Nijmegen, The Netherlands.

CONTENTS

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CHAPTER 1

INTRODUCTION

Many processes in health and disease represent the interaction between host (endogenous) and environmental (exogenous) factors. In this interplay, genetic factors play a major role. An important example of host interaction with exogenous factors in medicine is the patient's reaction to drug treatment. In this respect many data exist on drug toxicity, clinical response, and pharmacokinetics. However, the study of the influence of genetical factors on drug effects is still in its infancy, as indicated by the scarce data available. The present thesis reports studies aimed to detect genetical factors in drug response by correlating HLA phenotypes with the way patients with rheumatoid arthritis react to so-called second line drugs, a corner stone in the treatment of this disease. Before presenting the aim of the study in more detail, we would like to present in brief some relevant data:

- on the clinical syndrome rheumatoid arthritis
- on its therapy
- on how patients react to second line drugs in terms of effect and side effects
- on what was known about the genetics of both rheumatoid arthritis and reaction to second line drugs, at the moment this study started.

Rheumatoid arthritis: clinical features

Rheumatoid arthritis is a chronic systemic inflammatory disorder of unknown etiology characterized by the manner in which it involves joints. In addition to arthritis, which is often polyarticular and symmetrical, there are extra articular manifestations, of which the rheumatoid nodule is the best known example (1). General symptoms include fatigue, malaise, weakness and general aching and stiffness in part of the patients. Laboratory features include the presence of rheumatoid factors in the serum, anaemia and elevated sedimentation rate, the latter two abnormalities reflecting disease activity. The course of the disease is variable and capricious, ranging from episodes of polyarthritis, alternated by spontaneous remissions to a (rapidly) progressive arthritis, sometimes with widespread systemic features (2). This stresses the need for controlled observations in studies on rheumatoid arthritis (3). The prognosis in terms of validity is largely determined by joint involvement, the latter being largely dependent on the degree and rapidity of onset of destructive lesions in the joint and periarticular structures.

Treatment of rheumatoid arthritis

The plan of treatment may be considered under two headings. Firstly, treatment modalities concerning modification of the patient, including rest, drugs, physical therapy and surgery; and secondly, there may be a need for modification of the environment in terms of appliances, housing, occupation and transportation. It may appear from this compilation, that many disciplines can be involved in the treatment of a patient with rheumatoid arthritis. As far as drug treatment is concerned, several groups of drugs can be used. If pain is the only symptom, while no signs of inflammatory activity are present, simple analgesics may be used. In case of significant inflammatory activity, one or more of the following categories should be considered:

- non-steroidal anti-inflammatory drugs: e.g. salicylates, indomethacine, naproxen. These drugs give relief of pain and short term suppression of inflammation. They do not essentially alter the natural history of the disease.
- second line drugs: antimalarials (chloroquine compounds), gold salts, d-penicillamine and immunosuppressive drugs. These drugs possess the potency to induce a remission. Disadvantages of this category include thehigh incidence of toxicity, the delayed action (these drugs do not work until 2 to 3 months after institution of the therapy) and the fact that a number of patients do not respond to the treatment.
- Low dose steroids: this treatment is given when the above antirheumatic drugs are insufficiently effective or cause side-effects.

Gold salts and d-penicillamine

a. Preparation and dosage.

Gold salts are available in the form of parenteral gold and recently also of oral gold. The parenteral form, used in the Netherlands and therefore in our studies, is aurothioglucose (AuTG), a water soluble suspension in oil. The dose scheme generally used, is 50 mg AuTG per intramuscular injection per week during 20 weeks, after which the dose is reduced gradually. Laboratory controls include peripheral blood leucocyte count and differential, thrombocyte count and urinalysis for protein before each injection.

D-penicillamine (DP) is started in a daily dose of 250 mg according to the go low-go slow principle (4). If this dose is ineffective, monthly increments of 125 to 250 mg up to a maximal daily dose of 750 mg are given. Toxicity controls are essentially the same as for AuTG and are done weekly

in the first months. If an effective maintenance dose is reached, interval between controls for toxicity can be decreased to once per three to four weeks.

b. Efficacy.

Both drugs are generally not effective before three months of treatment, so that evaluation of efficacy before that period is not useful. Parameters to assess disease activity are manyfold. They can be divided into subjective ones, e.g. morning stiffness and pain; semi-objective parameters, e.g. joint tenderness and grip strength and objective ones such as erythrocyte sedimentation rate (ESR) and hemoglobin. These parameters can be used separately or as part of an index like those advocated by Mallya et al (5) or Van Riel (6). By quantitating the parameters and therefore the indices one can define the degree of response during therapy.

c. Toxicity.

Frequencies of toxicity during gold salts and DP therapy are given in table I. Most of the side effects occur during the first 6 months. It should be stressed that toxicity on gold, not necessarily predisposes the patient for (the same) toxicity on DP (7,8) (see Table II). This is relevant because DP is usually given after gold salt treatment. Several studies have indicated particular risk factors for the development of side effects on gold salts or DP (see table II). Since DP is also used for other indications, it became clear that rheumatoid arthritis perse is a risk factor leading to more frequent side effects. For gold salts similar data are not available.

TABLE I. Incidence of side effects on intramuscular gold and d-penicillamine in patients with rheumatoid arthritis.

type of reaction	gold salts (24) %	d-penicillamine (25) %
all reactions	20 - 39	37
mucocutaneous	15 - 30	12
proteinuria	3 - 7	9
leucopenia and thrombocytopenia	2	9
rare toxic reactions	cholestatic hepatitis enterocolitis pulmonary infiltrates bone marrow aplasia	bronchiolitis obliterans myasthenia gravis Goodpastures syndrome bone marrow aplasia

Table II. Factors that increase the risk for the development of toxic reactions on gold salts and d-penicillamine.

gold salts	d-penicillamine
high dosage(26)	rheumatoid arthritis (32)
water soluble preparations(27,28)	short term intervals between dose increments(4)
IgA deficiency(29)	age beyond 60 years(33)
presence of IgE antibodies to gold(30)	impaired sulphoxidation(34)
HLA B8-DR3(21) DR2(20)	HLA DR3(21) DR4(35)
IgM rheumatoid factor negativity (proteinuria)(31)	History of gold salt induced toxicity (36)

Genetical factors and rheumatoid arthritis

Studies in the pre-HLA era have indicated that genetic factors are important in the development of rheumatoid arthritis. These studies include multiple case families and twin studies (9). HLA studies have indicated an association between rheumatoid arthritis and the antigen Dw4 and DR4, at least in clinical patient populations (10). This association, however, was not found in a population survey (11). In addition to this association, several studies have mentioned an association between certain HLA antigens and the presence of certain extra-articular manifestations, like Sjögren's syndrome, Felty's syndrome and vasculitis (12,13,14). Family studies using HLA typing have so far not consistently shown segregation with one particular haplotype or antigen (15). Haplotype sharing did not reveal deviation from the expected distribution (16,17,18). At this moment no consensus exist whether HLA antigens are associated with seropositivity/seronegativity or a worse prognosis (10).

Genetics of toxicity of antirheumatic drugs

At the start of the present study only few data were available on the effect of genetic factors on the development of toxicity of antirheumatic drugs. The first report was an asociation between levamisole-induced granulocytopenia and the HLA antigen B27 (19). As far as gold salts and d-penicillamine are

concerned, the first report was that of Panayi et al (20), who showed an association between toxic reactions on these drugs and the HLA phenotypes DR2 and DR3. In 1980 Wooley et al demonstrated an impressive association between aurothiomalate-induced proteinuria and HLA B8 DR3 (21). There was no significant association between DP-induced proteinuria and the latter antigens. A study by Latts et al suggested an increased susceptibility for gold-induced toxicities in patients positive for the HLA antigen B12 (22). Finally, in 1981 Coblin et al (23) showed a clearly increased frequency of HLA DR3 in patients with aurothiomalate- or AuTG-induced thrombocytopenia.

Aim of the study

The present thesis reports studies on genetical factors influencing the patients reaction to two second line drugs using HLA antigens as a genetic marker system. The two second line antirheumatic drugs studied are AuTG, the parenteral gold preparation used in this country (the preparation in use in Anglo-Saxon countries is aurothiomalate), and DP. We looked for genetic influence not only on the development of toxicity, but also on type of clinical response to the drug, both in groups of unrelated RA patients and in families with multiple cases of RA.

By means of a case control study we investigated the association between HLA phenotypes and the development of proteinuria after AuTG or DPtreatment. Not only the development of proteinuria perse, but also its degree and time of onset were taken as variables (chapter 2).
Since this study indicated differences in HLA-phenotype associations between AuTG- and DP-induced proteinuria, we investigated whether previous AuTG-induced proteinuria is a risk factor for developing proteinruia during subsequent DP treatment as stated by some authors (37,38) (chapter 3).

The most severe toxicities of both AuTG and DP are hematotoxic side effects. In chapter 4 we report data on HLA associations in AuTG- and DP-induced thrombocytopenias and leukopenias obtained in a case control study.

Only scanty information exists on whether there is a genetic predisposition to a certain type of clinical response (excellent-, moderate- or non-response) to second line antirheumatic drugs. Chapter 5 reports data on HLA phenotype frequencies in groups with different types of response to AuTG treatment, obtained in a large cohort study.

In the final part of this thesis we studied the reaction to either AuTG or DP in first degree relatives with RA treated with the same second line drug. The larger part of these patients were obtained from a large study project. Our studies were initiated by the observation of a remarkably similar reaction to AuTG therapy, both in terms of toxicity and clinical response, in two pairs of sibs, who proved to be HLA identical (chapter 6). Subsequently we collected 13 pairs of first degree relatives with identically treated RA and studied concordances in terms of toxicity and clinical response to drug treatment. In addition we studied the influence of HLA-haplotype sharing and sex on concordancy (chapter 7). Details of the multiple case families mentioned above are given in the Addendum.

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CHAPTER 2

HLA-DR ANTIGENS AND PROTEINURIA INDUCED BY AUROTHIOGLUCOSE AND D-PENICILLAMINE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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HLA-DR Antigens and Proteinuria Induced by Aurothioglucose and D-Penicillamine in Patients with Rheumatoid Arthritis

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Abstract **By means of a case-control study we investigated the association between HLA phenotypes and the development of proteinuria after aurothioglucose or D-penicillamine treatment in patients with rheumatoid arthritis (RA) HLA-DR3 was markedly increased in 44 treatment cases compared with 66 RA controls (46 versus 18 , p = 0 002) HLA DR3 positive patients were at greater risk during treatment with D-penicillamine (RR 10 1, p = 0 001) than gold treated cases (RR 1 7, p 0 365) The associations between HLA-DR3 and nephrotic syndrome (RR = 6 3, p = 0 004) and early onset proteinuria (RR = 5 4, p < 0 001) were stronger compared with uncomplicated proteinuria (RR = 3 1, p = 0 017) and late-onset proteinuria (RR = 1 6, p 0 459), respectively It appears that genetic factors in RA influence the development, the degree and the time of onset of drug induced proteinuria (*J Rheumatol 10* 948-953, 1983)**

Key Indexing Terms
RHEUMATOID ARTHRITIS HLA ANTIGENS PROTEINURIA
AUROTHIOGLUCOSE IMMUNOGENETICS D PENICILLAMINE

Patients with severe rheumatoid arthritis (RA) treated with antirheumatic drugs such as aurothioglucose (GTG) and D-penicillamine (DP) is often complicated by toxic reactions sometimes necessitating discontinuation of the drug Mechanisms underlying the development of toxic reactions are obscure, and may not necessarily be the same for different toxicities A comparison of patients with Wilson's disease and RA receiving DP showed a higher frequency of toxic reactions in patients with RA[1], suggesting that patients with RA are more prone to drug toxicity

Results of several recent studies have suggested a genetic basis for at least some toxic reactions to antirheumatic drugs in patients with RA Veys, *et al*[2,3] reported that the possession of the HLA-B27 antigen correlates with an increased risk of granulocytopenia in patients with RA taking levamisole In addition, Panayi *et al*[4] showed that RA patients positive for HLA-DR3 or DR2 had an increased risk of toxic manifestations during treatment with sodium aurothiomalate (GSTM) or DP

Recently Wooley, *et al*[5] reported an increased frequency of HLA-DR3 in patients with proteinuria taking GSTM or DP They calculated a relative risk of developing proteinuria during GSTM therapy of 32 in these DR3 positive patients Since a relative risk of this order of magnitude may have practical consequences, we studied a large series of RA patients with gold- or DP-induced proteinuria seen at our hospitals In this case-control study we looked for possible associations between the development of proteinuria during GTG or DP therapy and HLA antigens

MATERIALS AND METHODS

Patients Forty four patients with definite or classical RA according to the ARA criteria[6] developed proteinuria either on GTG or on DP or on both were entered into study Data were collected from laboratory check lists and clinical notes on patients treated during the past 10 years

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at the University Hospital and St Maartenskliniek Nijmegen (28 patients) and at the Ziekenzorg Hospital, Enschede (16 patients) both cities situated in the eastern part of the Netherlands A 1st control group consisted of 66 patients with definite or classical RA who had been treated for at least 12 months with either drug without signs of proteinuria on routine testing A 2nd control group consisted of 277 healthy blood donor volunteers randomly selected and typed over the same period as the RA patients Characteristics of both patient groups are shown in Table 1 Extraarticular manifestations such as nodules, Sjogren's syndrome, vasculitis, polyserositis and Felty's syndrome occurred in 58 patients and were more frequent in the proteinuric group A positive family history for RA obtained by direct questioning was equally frequent in patients and RA controls

Drug regimen and toxicity GTG (50 mg IM) was given weekly to a cumulative dose of 1000 mg, the dosage was then reduced to a maintenance dose of 50 mg/4 weeks DP was prescribed in an initial dose of 250 mg/day with increments up to 1000 mg in some depending on the clinical response Monitoring for side effects (physical examination, leukocyte count and differential, platelet count and routine testing for proteinuria) was initially done weekly and later on during maintenance therapy at least once/6 weeks Proteinuria found on routine testing was quantitated in g/24 h (28 patients) or g/l (16 patients) The criterion for inclusion in the patient group was proteinuria of more than 1 g/24 h or 1 g/l The proteinuria was considered to be drug induced only if it reversed after discontinuation of therapy For inclusion in the RA control group, routine tests had to show no proteinuria for at least one year Of the 44 proteinuric patients studied, 22 were GTG and the other 22 DP induced Twelve of the proteinuric patients fulfilled the criteria for nephrotic syndrome Rectal biopsies of 5 of these cases showed no deposition of amyloid Glomerular function as indicated by serum creatinine was unaffected in all cases but proteinuria persisted for months after discontinuation of therapy and in some cases proteinuria was still traceable after one year

HLA typing Typing for HLA-A, B, and C antigens was done according to Mittal *et al*[7] by standardized NIH microlymphocytotoxicity techniques, using 118 sera for all established HLA-A B and C antigens, including BW4 and BW6 and low frequency W specificities (class I antigens) HLA-DR typing was done on enriched B lymphocyte suspensions obtained after rosetting with ALT treated sheep red blood cells[8] The 58 test sera for typing antigens DR1 through DRW10 (class II antigens) were partly of local origin and partly obtained by national and international exchange all sera being standardized against the 8th International Histocompatibility Workshop sera[9] and also during the Dutch B cell Typing Work Shop (1981)

Statistical analysis The increase in risk associated with certain HLA types was expressed as a relative risk (RR) estimated from the case control data by means of the exposure odds ratio[10] To evaluate whether the RR estimate differed significantly from unity (its expected value under the null hypothesis of no association), we calculated the Mantel-Haenszel Chi-square statistic[11], and obtained 2-sided p values Finally, we computed 95% confidence limits of the RR estimate[12] to give an impression of the statistical uncertainty involved In the tables we use the following notation to distinguish the relative risk estimates associated with a certain HLA antigen (indicated in brackets) among the cases and the 2 control groups RR_{CR} is the relative risk when cases are compared to RA controls, RR_{CH} is the relative risk when cases are compared to healthy controls, and RR_{RH} is the relative risk when RA controls are compared to healthy controls RR_{CR} gives contrasts in HLA antigen that are associated with the development of proteinuria to the drugs studied RR_{CH} compares RA patients who developed proteinuria with the healthy population, while RR_{RH} compares the other RA patients (those not developing proteinuria to the drugs studied) with the healthy population

Table 1 *Clinical characteristics of the RA patients (n = 44) and RA controls (n = 66)*

	Cases	RA Controls
Age (median)	58	60
Male female ratio	1 3	1 3
IgM rheumatoid factor positive (%)	95	95
ANA positive (%)	52	40
Extraarticular manifestations (%)	68	41
Family history positive (%)	29	28

RESULTS

Table 2a compares HLA-DR gene products in patients, RA controls and healthy controls The different calculable RR estimates of interest are tabulated in Table 2b The frequency of antigen DR4 was significantly increased in both patients and RA controls as compared with healthy controls, while at the same time the prevalence of DR4 did not significantly differ between patients and RA controls Antigen DR3 was significantly increased in patients as compared with RA controls, while at the same time the prevalence of DR3 did not differ significantly between RA controls and healthy controls

Table 3 shows the association between antigen DR3 and proteinuria for all patients and for GTG and DP-induced proteinuria, respectively A higher RR regarding this antigen was observed for DP than for GTG When the presence of HLA-DR3 was compared between patients with nephrotic syndrome and those with uncomplicated proteinuria, the RR was higher in the former than in the latter group (Table 4) In addition, DR3 positive cases developed proteinuria earlier than did DR3 negative cases (Table 5)

Table 2a *Distribution of HLA-DR antigens in RA patients, RA controls and healthy controls*

HLA-DR Antigen	RA Patients (n = 44) No	%	RA Controls (n = 66) No	%	Healthy Controls (n = 277) No	%
DR 1	11	26	21	32	70	25 3
2	7	16	12	18	89	32 1
3	20	46	12	18	64	23 1
4	20	46	36	55	54	19 6
5	6	14	15	23	65	23 5
6	9	21	12	18	73	26 4
7	5	12	9	14	55	20 0
8	2	5	3	4	16	5 7
9	1	2	3	4	8	3 0
10	0	0	2	3	2	0 7

Table 2b. *Estimates of the relative risks regarding antigens DR3 and DR4**

	Point Estimate	95% Confidence Interval	p-Value (2-sided)
RR_{RH} (DR 3 +)	0 7	0 4 - 1 5	0 388
RR_{CH} (DR 3 +)	2 8	1 5 - 5 2	0 002
RR_{CR} (DR 3 +)	3 8	1 6 - 8 7	0 002
RR_{RH} (DR 4 +)	5 0	2 9 - 8 5	<0 001
RR_{CH} (DR 4 +)	3 4	1 8 - 6 5	<0 001
RR_{CR} (DR 4 +)	0 7	0 3 - 1 5	0 352

* Notation, see Materials and Methods

Table 3 *Association between proteinuria induced by GTG or DP and antigen HLA-DR3 Results obtained from 44 RA patients and 66 RA controls*

Drug	DR 3	RA Patients (n = 44) No	RA Controls (n = 66) No	Relative Risk (DR 3) Point Estimate	95% Confidence Interval	p Value
GTG or	+*	20	12	3 7	1 6 - 8 7	p = 0 002
DP	−**	24	54			
GTG	+	7	9	1 7	0 5 - 5 5	p = 0 365
	−	15	33			
DP	+	13	3	10 1	2 5 - 40 3	p = 0 001
	−	9	21			

* + present
** − absent

Table 4 *Frequency of antigen DR3 in RA patients with and without nephrotic syndrome (NS)*

	RA Patients		
	NS Present* n = 12	NS Absent n = 32	RA Controls n = 66
Antigen DR 3			
Present	7	13	12
Absent	5	19	54
RR_{CR}	6 3	3 1	
95% CI	1 8 21 3	1 2 7 8	
p Value	0 004	0 017	

* This category includes 4 NS induced by GTG and 8 NS induced by DP

Frequencies of HLA-A, B, and C antigens did not differ significantly between patients RA controls and healthy controls except for antigen B8 in the patients, which reflects the linkage disequilibrium with antigen DR3 Of those patients and healthy controls that were HLA-DR3 positive, 17 out of 20 patients (85%), 8 out of 12 RA controls (66%), and 43 out of 64 healthy controls (67%) also were B8 positive On the other hand, 17 out of 21 patients (82%), 8 out of 17 RA controls (50%) and 45 out of 64 healthy controls (70%) that were HLA B8 positive also possessed antigen DR3 Although the combined occurrence of antigens B8-DR3 was found more frequently in the patient group, this frequency was not statistically different from that observed in RA controls and healthy controls Antigen DR2 was decreased in both patient groups as compared with healthy controls, at a statistically significant level ($p = 0\ 02$ with correction for the number of DR antigens tested for)

DISCUSSION

Our case-control study shows an increased frequency of HLA-DR3 in patients developing proteinuria on GTG and DP as compared with the non-proteinuric controls The strongest association between the presence of HLA-DR3 and proteinuria existed in the DP treated cases and in those who presented with nephrotic syndrome

Table 5 *Relation between early onset proteinuria (within one year after institution of therapy) and antigen DR3*

	RA Patients		RA
	Early Onset	Late Onset	Controls
DR3 Present	16	4	12
Absent	13	11	54
RR	5 4	1 6	
95% CI	2 2 14 0	0 4 6 0	
p value	<0 001	0 459	

Wooley, *et al*[9] reported that the RR of developing of proteinuria on GSTM in an HLA-DR3 positive person would be as high as 32 We could not confirm this high RR estimate In our study the RR associated with GTG was 1 7, and it was not statistically significant at the 5% level By contrast, in our data the RR estimate with DP was 10 1 and was statistically highly significant This RR estimate is somewhat higher than the 4 5 reported by Wooley *et al*[9] and the 3 9 by Stein, *et al*[13] Our overall RR (both modes of treatment) was 3 7, and statistically significant at the 5 % level Although our case series was about twice as large as that reported by Wooley, *et al*[9] which will in principle result in more stable estimates the fact remains that the separate tabulation of the effects of HLA-DR3 on each of the drug regimens results in small numbers in the cells of the 4-fold tables Most likely, it is the smallness of these numbers that is responsible for the widely divergent RR estimates To give an impression of the rather wide ranges of the estimates that result from our data, we have reported in the tables the 95% confidence intervals of the estimates as well Another possible factor contributing to the discrepant findings may be the use of different gold preparations — GSTM versus GTG in our series Both preparations possess the aurosuphur group attached to either malate or glucose The different physical properties are determined by the hydrophylic groups both preparations are soluble in water, but only GTG can be suspended in oil Water soluble preparations are more rapidly absorbed after injection, resulting in immediate higher blood levels and consequently a larger urinary loss of gold shortly after administration These peak levels are avoided by using preparations suspended in oil such as GTG It is conceivable that these mechanisms play a role in the observed significantly increased incidence of toxic reactions including proteinuria during GSTM administration as compared with GTG[14]

Possible mechanisms underlying the association between DR3 and gold and DP induced proteinuria are still obscure Renal biopsy studies from gold or DP induced proteinuria have indicated that the lesion is probably a membranous type immune complex (IC) glomerulonephritis[15,16] Recent

studies have shown an increased frequency of HLA-DR3 in several diseases, e g , type I diabetes mellitus, Sjogren's syndrome, membranous glomerulonephritis, Graves' disease, systemic lupus erythematosus (SLE), chronic active hepatitis, celiac disease, myasthenia gravis and dermatitis herpetiformis[1] In addition, RA patients positive for the HLA-DR3 antigen show more extra-articular manifestations[4] Since the above mentioned diseases are generally thought to be of autoimmune origin, the presence of HLA-DR3 may predispose to autoimmunity and/or IC disease One possible explanation may be that gold salts and DP act as a hapten that leads to autoantibody formation and possibly to IC disease Another intriguing possibility is suggested by a recent study on patients with dermatitis herpetiformis[18] In this study it was shown that 50% of the patients, all positive for antigen B8 or DR3 or both, had a delayed Fc receptor mediated clearance of IgG-sensitized autologous erythrocytes as compared with normal controls A similar defect was also found in half of the healthy controls positive for HLA-B8/DR3 In this respect it may be relevant that 85% of our DR3 positive patients also possessed the HLA-B8 antigen, whereas only 66% of the RA controls and 67% of the healthy controls positive for DR3 were also HLA-B8 positive If Fc receptor-mediated clearance is indeed delayed in patients having the HLA-B8/DR3 antigens, then accumulation of GTG in the reticuloendothelial system, which has been demonstrated[19], could accentuate this defect, leading to more circulating IC and eventually to IC disease

Other, as yet unexplored factors, may also be important This is well illustrated by another example of drug induced disease, i e , hydralazine induced SLE Drug metabolism seems to be a crucial factor in its pathogenesis, since the disease occurs more frequently in slow than in rapid acetylators[20] In addition, this is a good example of a drug induced disease with more than one contributing factor A recent study[21] demonstrated that a combination of female sex, DR4 positivity and slow acetylatorship increases the risk of hydralazine induced SLE up to 100% That different mechanisms may be operative in the various side effects of one drug is also indicated by the fact that Wooley, *et al*[5] were unable to demonstrate associations between other toxic effects on GTG and DP and HLA-D antigens Preliminary data from our group confirm this observation, at least so far as gold induced rashes are concerned In contrast we observed 2 cases presenting with severe gold induced thrombocytopenia and 2 additional cases with severe and recurrent granulopenia on both gold and DP All 4 patients were positive for antigen DR3 Coblin, *et al*[22] found a strong association between thrombocytopenia induced by GTG and antigen DR3 Recently, Bardin, *et al*[23] reported on the positive association of toxicity secondary to a different gold salt, aurothiopropanololsulphate, and antigen DR3 A minority of these patients presented with proteinuria and hematologic side effects

Our results seem to suggest that patients with HLA-DR3 are at higher risk for developing proteinuria during DP therapy We feel that these observations need confirmation Prospective studies should be done to determine the actual risk in this phenotypic subset Meanwhile, in our opinion there is no firm reason to perform tissue typing before DP is started This is because the associations found are definite but not very strong, the alternatives in drug treatment are limited, and drug induced proteinuria is reversible and very rarely leads to renal function impairment

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CHAPTER 3

THE RELATIONSHIP BETWEEN AUROTHIOGLUCOSE- AND D-PENCILLAMINE-INDUCED PROTEINURIA

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The Relationship between Aurothioglucose- and D-Penicillamine-Induced Proteinuria

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We studied patients with rheumatoid arthritis who have been treated with aurothioglucose (Au) and subsequently with D-penicillamine (DP), and who developed drug-induced proteinuria, over a 10-year period. Twelve patients developed Au-induced and 19 DP-induced proteinuria. Of the 12 patients with Au-induced proteinuria, only 2 (17%) developed DP-induced proteinuria, indicating a slightly increased risk as compared with the overall incidence (9.3%) of this reaction in 168 DP-treated patients. In addition, only a minority (2 out of 19, 10.6%) of patients with DP-induced proteinuria had previous Au-induced proteinuria. These data may indicate that different mechanisms are operative in Au and DP-induced proteinuria, as is also suggested by the finding that HLA-DR3 was present more frequently in the latter (50%) than in the former (21%). A history of previous Au-induced proteinuria is insufficient reason to deny these patients the benefits of subsequent treatment with DP.

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In recent years D-penicillamine has become recognized as a major antirheumatic agent (15, 25). The drug is frequently used after gold therapy has caused toxic reactions or proved ineffective. The frequency of side effects of D-penicillamine is comparable to that of intramuscular gold and the toxic reactions to both drugs show a considerable overlap (4, 6). From a practical point of view it would be important to know whether patients whose severe toxic reactions to gold necessitate discontinuation of the drug, run a greater risk of developing similar toxic reactions to D-penicillamine. Data on drug-induced proteinuria have so fare been contradictory. Several studies suggest that patients with D-penicillamine-induced proteinuria frequently have a history of gold-induced proteinuria (7, 3, 6, 19), whereas others have been unable to find such an association (13, 27).

In a recent study we looked for associations between HLA phenotypes and aurothioglucose- or D-penicillamine-induced proteinuria in patients with rheumatoid arthritis developing this type of side effect (20). We found HLA-DR3 to be significantly increased in patients with D-penicillamine-induced proteinuria, but not in those with aurothioglucose-induced proteinuria, suggesting a difference in the pathogenetic mechanism involved. We then studied the relationship between aurothioglucose- and D-penicillamine-induced proteinuria by selecting from the above series those proteinuric patients who had been treated with both drugs, in all cases D-penicillamine treatment following treatment with aurothioglucose.

This should inform us about the number and clinical characteristics of patients who developed proteinuria on both drugs and therefore give us an estimate of the recurrence

rate of proteinuria in D-penicillamine-treated patients who had previously developed this type of side effect on aurothioglucose. Our results indicate that patients with aurothioglucose-induced nephropathy run only a slightly increased risk of developing D-penicillamine-induced proteinuria.

Selection of patients

Patients for the present study were selected from a larger series of 44 patients with classical or definite rheumatoid arthritis (RA) (14) and drug induced proteinuria studied for other purposes and reported on elsewhere (20). This series included all patients with RA and proteinuria (see below) on aurothioglucose (Au), D-penicillamine (DP), or both, seen at two centres for rheumatic diseases in the eastern part of The Netherlands (Nijmegen and Enschede) between 1972 and 1982.

For the present study we selected from this series only those (29 out of 44) who had been treated with both drugs, in all instances DP after Au. This group of patients would allows us to study the frequency of proteinuria on DP after Au-induced proteinuria and would also inform us about the frequency of earlier Au induced proteinuria in patients who developed DP induced proteinuria. The criterion for inclusion was proteinuria of more than one gramme per 24 hours or one gramme per litre. The proteinuria was considered to be drug induced if it reversed after discontinuing the therapy. Patients with pre-existent renal disease, chronic urinary tract infections or longstanding diabetes mellitus were not included.

If it is stated that either of the two drugs had not induced proteinuria, this means that this particular drug had been used for at least one year without development of proteinuria, or that the drug had to be discontinued in view of other toxic reactions or ineffectiveness (usually after 6 months of use). Since we had to compare the frequency of proteinuria on DP in patients with previous Au induced proteinuria vis à vis that in an unselected group of DP-treated patients, we calculated the latter from the number of DP-induced proteinurias ($n=16$) as a percentage of all patients who had received the drug between 1972 and 1982 ($n=172$) at the centre for rheumatic diseases in Nijmegen.

Dose regimen and drug monitoring

Aurothioglucose (50 mg i.m.) was given once a week up to a cumulative dose of 1000 mg, thereafter the interval was prolonged to 2–4 weeks. D-penicillamine was prescribed in an initial daily dosage of 250 mg. Increments were made according to the go-slow–go-low regimen (8) up to 750–1000 mg in some. Urinalysis for proteinuria was done using the dip-stick method, initially weekly and subsequently once every 4–6 weeks. If proteinuria was detected the amount was quantitated in grammes per 24 hours or per litre.

HLA typing

Typing for HLA ABC antigens was done according to Mittal et al. (12) by standardized NIH microlymphocytotoxicity techniques, using 118 sera for all established HLA ABC specificities including Bw4, Bw6 and low frequency W specificities. HLA DR typing was done on enriched B-lymphocyte suspensions obtained after rosetting AET-treated sheep red blood cells (9). The 58 test sera for typing antigens DR1 through DRw10 were partly of local origin and partly obtained by national and international exchange, all sera being standardized against the 8th International Histocompatibility Workshop sera (26) and also during the Dutch B-cell typing Workshop in 1981.

Statistical methods

The relative risk of developing proteinuria on D-penicillamine in a patient with a history of Au induced proteinuria was computed together with 95% confidence interval by the calculator programs of Rothman & Boice (16). The 95% confidence interval gives an estimate of the statistical uncertainty which is involved in the relative risk estimation, if unity falls outside the 95% confidence interval the relative risk is statistically significant at the 5% level.

RESULTS

Recurrence of proteinuria

Proteinuric patients could be divided into three groups: those with proteinuria on Au, but not on DP (Au+ DP−), those with proteinuria on DP but not on Au (Au− DP+), and those with proteinuria on both drugs (Au+ DP+) (see Table I). Thus, 2 of the total of 12 Au-induced proteinurias (17%) recurred on DP, whereas 10 (83%) did not. Of the latter 10

patients, 7 had to discontinue DP after 14 to 84 months 2 because of loss of benefit and 5 because of intestinal complaints Three patients are still using DP Table I also shows that 2 of the total of 19 patients with DP-induced proteinuria (9%) previously had Au-induced proteinuria One of these patients developed clinical nephrotic syndrome during both modes of treatment In the remaining 17 patients Au was stopped after 6 to 120 months for the following reasons rashes (8 patients), loss of initial benefit (7 patients) and prolonged remission (2 patients) Nephrotic syndromes were more frequently found in the DP-induced proteinurias (7 out of 19) than in the Au-induced proteinurias (3 out of 12) The same applies to the frequency of HLA-DR3, but HLA-DR4, seropositivity and extra-articular manifestations were equally distributed among the groups (Table I)

Table II shows data on our proteinuric patients and controls and data from the literature Our results indicate that the frequency of DP-induced proteinuria after Au-induced proteinuria is 17%, as compared with an overall incidence of 9 3% This gives a relative risk of 1 7 with 95% confidence intervals between 0 5 and 7 1

DISCUSSION

We determined the risk of proteinuria as a toxic reaction to D-penicillamine in RA patients who had previously suffered from aurothioglucose-induced proteinuria The incidence of D-penicillamine-induced proteinuria in these patients was 17% (2/12) and turned out to be only slightly higher than the overall incidence of proteinuria during D-penicillamine treatment (9 3%) Although the higher frequency (a relative risk of 1 7, i e almost twice as high) may suggest that D-penicillamine-induced proteinuria is more likely to occur in the patient with a history of Au-induced proteinuria, this estimate is not significant at the 5% level and carries a wide confidence interval (0 5 to 7 1) due to the small number of observations Evidently, as our findings demonstrate, the majority of patients with a history of proteinuria on gold are able to use D-penicillamine later on, indicating that the predictive value and consequently the clinical relevance of this anamnestic information is limited In this context it should be realized that proteinuria caused by either of these drugs nearly always takes a benign and self-limiting course From these data it seems

Table I *Clinical data and HLA DR antigen frequencies of 29 proteinuric patients*

Subset[a]	No of patients	First signs of proteinuria (months)	Nephrotic syndrome	Disease manifestations	HLA DR antigen frequencies (no of patients)				
					DR1	DR2	DR3	DR4	DR5–10
Au^+DP^-	10	2–12	2	ANA (4) IgMRF (10) nodules (8) Sjogren (1) vasculitis (1)	1	2	3	6	7
Au^+DP^+	2	2–3	1[b]	ANA (2) IgMRF (2) nodules (1) Sjogren (1) vasculitis (2)	–	–	1	2	–
$Au\ DP^+$	17	2–18	6	ANA (9) IgMRF (16) nodules (12) Sjogren (3) vasculitis (1)	3	2	8	6	11

[a] Au^+DP^-, patients with proteinuria on aurothioglucose but not on D-penicillamine, Au^+DP^+, patients with proteinuria on both aurothioglucose and D-penicillamine $Au\ DP^+$, patients with proteinuria on D-penicillamine but not on aurothioglucose — [b] See text

justifiable to treat RA patients with D-penicillamine when gold has to be discontinued because of proteinuria

Data published on this subject have so far been controversial (Table II) (3, 4, 6, 7, 10, 13, 19, 21, 22, 25, 27). Observations reported by Billingsley & Stevens (3), Halla et al (6) and Smith et al (19) suggest an increased risk of D-penicillamine induced proteinuria in patients with previous gold-induced proteinuria, but Webley & Coomes (27) found no such association. However, it should be realized that the studies summarized in Table II are not readily comparable as there are major differences between them. Important in this respect are the variations in the percentages of patients previously treated with gold and in the criterion for drug-induced proteinuria. Regarding the latter, it is interesting that the studies of Billingsley (3) and Halla (6), suggesting an increased risk of recurrence, both used a lower inclusion criterion for proteinuria (0·4 g) than Webley's study (27) and our own study (>1 g), we were both unable to demonstrate a significantly increased risk. The question arises whether inclusion of minor proteinuria increases the risk of including non-drug-related proteinuria. This is not inconceivable, since mild glomerular pathology unrelated to gold or D-penicillamine has been demonstrated in patients with rheumatoid arthritis (28). Moreover, minor but variable proteinuria often follows significant gold-induced proteinuria sometimes for a considerable time (18), and may be mistaken for D-penicillamine-induced proteinuria.

The fact that the overlap between gold- and D-penicillamine-induced proteinuria is only small, suggests that different mechanisms are operative in proteinuria induction by the two

Table II *Incidence of proteinuria induced by D penicillamine with reference to previous gold induced proteinuria*

Group	No. of patients	Incidence of proteinuria induced by D penicillamine *N* (%)	Previous treatment with gold (%)	Recurrence of proteinuria *N* (%)	Definition of drug induced proteinuria
Present series[a]					
All patients with gold-induced proteinuria with D-penicillamine later on	12	2 (17)			>1 g
All patients treated with D-penicillamine	172	16 (9·3)			
Previous series					
1974 multicentre trial group	54	9 (17)	54	Not stated	+ dipstick
1977 Tsang et al	44	2 (5)	100	1 (50)	>2 g
1979 Webley et al	144	11 (10)	65	None (0)	>1 g
1979 Stein et al	330	Not stated	69	4 (?)	Not stated
1980 Stein et al	259	19 (7)	59	Not stated	>0·5 g
1980 Hylland et al	56	3 (5)	84	2 (70)	Not stated
1980 Dodd et al	155	Not stated	19	3 (7)	Not stated
1981 Billingsley et al	25	6 (24)	84	5 (83)	>0·4 g
1982 Kean et al	114	13 (11)	47	3 (23)	+ dipstick
1982 Smith et al	405	20 (5)	38	4 (40)	Not stated
1982 Halla et al	90	14 (16)	100	8 (57)	>0·4 g
1982 Steven et al	44	5 (11)		Not stated	>0·4 g

[a] Including all registrations between 1972 and 1982

drugs Two of our observations support this Firstly, in previous studies we (20) and others (23) have shown that D-penicillamine-induced (but not gold-induced) proteinuria is strongly associated with HLA-DR3 This observtion is at variance with other studies (29, 5), probably due to differences in patient selection and/or the use of a different gold salt, i e aurothiomalate instead of aurothioglucose (11) Secondly, clinical nephrotic syndromes are more frequent in the D-penicillamine- than in the gold-induced proteinuria group This observation confirms those of others (1) It should be borne in mind that interpretations of such data may be biased to some extent by patient selection, since patients receiving D-penicillamine frequently have a history of gold intolerance The HLA haplotype A1 B8 Cw7 DR3 is found more frequently in patients with gold toxicity (2), suggesting that the proportion of these antigens may be higher in patients treated with D-penicillamine than in patients who receive the first course of gold

On the other hand, we cannot exclude the possibility that the above-mentioned differences are merely an expression of differences in dose regimen of the two drugs, since clinical experience suggests a dose dependency of gold- and D-penicillamine-induced proteinuria, in addition, the glomerular abnormality most commonly found is the same in both gold- and D-penicillamine-induced proteinuria, i e membranous type glomerulonephritis On the other hand observations made by Samuels et al (17) seem to suggest that this type of glomerular lesion may be aetiologically associated with rheumatoid arthritis, usually presenting with mild symptoms

From a clinical point of view, it appears from these retrospective data that gold-induced proteinuria is insufficient reason to deprive a patient with rheumatoid arthritis of further treatment with D-penicillamine Moreover, our data do not warrant an increase in the frequency of regular check-ups for proteinuria during treatment in cases of previous gold-induced proteinuria

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CHAPTER 4

HLA ASSOCIATIONS IN AUROTHIOGLUCOSE- AND D-PENICILLAMINE-INDUCED HAEMATOTOXIC REACTIONS IN RHEUMATOID ARTHRITIS

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HLA associations in aurothioglucose- and D-penicillamine-induced haematotoxic reactions in rheumatoid arthritis

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HLA phenotype frequencies were studied in 21 rheumatoid arthritis (RA) patients with haematotoxic reactions to aurothioglucose (AuTG) or D-penicillamine (DP), 65 matched RA controls and 277 healthy controls.

Antigens B8 and DR3 were significantly increased in the toxic RA patient group as compared to both RA controls and healthy controls. These contrasts were strongest in the patients with AuTG-induced thrombocytopenia or leucopenia: all patients developing either reaction to this drug were B8- and/or DR3-positive ($p < 0.001$), 7 (78%) being positive for both antigens. In the patient group with DP-induced reactions these antigens were also increased but these differences were not significant. In the latter group the prevalence of antigen DR4 was high, especially in the patient group with DP-induced thrombocytopenia: all 12 patients with this type of reaction being DR4 positive.

Our data suggest that haematotoxic reactions to AuTG and DP develop primarily (or even exclusively) *in genetically predisposed RA patients*. Furthermore, the HLA phenotype contributing to an increased risk seems not to be the same for the two anti-rheumatic drugs studied.

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Patients with severe rheumatoid arthritis (RA) are often treated with disease modifying drugs like aurothioglucose (AuTG) and D-penicillamine (DP). However, treatment with these drugs is frequently complicated by side effects, including dermatitis, proteinuria and haematotoxic reactions, often necessitating discontinuation of the drug. Recent studies have indicated an association between HLA-DR3 positivity and the risk of developing proteinuria on aurothiomalate (Gran et al. 1983, Wooley et al. 1980) and D-penicillamine (Speerstra et al. 1983). Since only a minority of the HLA-DR3-positive RA patients develop proteinuria and since proteinuria is nearly always reversible and seldom a serious life-threatening condition, this association is at the moment of little practical value. On the other hand, haematotoxic reactions to AuTG and DP are serious and sometimes life-threatening. The available information on HLA associations with these reactions is scanty (Co-

blin et al 1981) In the present study we report on HLA associations with AuIG- and DP-induced haematotoxic reactions and their possible practical implications

Material and methods

Selection of patients Records of all patients with classical or definite rheumatoid arthritis (RA) attending the Department of Rheumatology between 1976 and 1983 were reviewed for thrombocytopenia or leucopenia during treatment with aurothioglucose (AuTG) or D penicillamine (DP)

Twenty one Caucasian patients (12 females 9 males) were identified varying in age between 23 and 74 years (median 54 years) at the time of registration of the side effect

Table 1 lists the time of onset after the start of treatment and the severity of the reaction Seventeen patients had one type of reaction Patients 19 and 20 had a simultaneous fall of leucocytes and thrombocytes on AuIG Patient 18 had agranulocytosis on AuIG and subsequently leucopenia after switching to DP and patient 19 had leucopenia on AuTG and thrombocytopenia on DP In summary there were 25 reactions 6 leucopenias (4 on AuIG 2 on DP) 19 thrombocytopenias (7 on AuIG 12 on DP) and with reference to exposure there were 11 AuIG-induced and 14 DP induced reactions

Table 1
Clinical details and HLA phenotypes of 21 RA patients with hematotoxic reactions on AuIG[1] and DP

Patient	Exposure	Reaction	HLA phenotype
1	AuTG 7 months	TP[3] 7 ×10 /l	A1 B8 B12 DR1 DR3
2	3 weeks	5 ×10 /l	A1 A2 B8 B40 DR3 DR5
3	20 months	80 ×10 /l	A2 A3 B8 B15 DR3 DR4
4	9	30 ×10⁹/l	A1 A32 B7 B8 DR2 DR3
5	4	5 ×10 /l	A2 B8 B40 DR3 DRw8
6	DP 16 months	LP[4] 1 7×10 /l	A1 A2 B8 B40 DR1 DRw6
7	2	TP 74 ×10 /l	A1 A2 B8 B12 DR4 DR5
8	5	63 ×10 /l	A1 A9 B8 B15 DR3 DR4
9	4	120 ×10⁹/l	A1 B8 DR4 DRw6
10	6	84 ×10 /l	A1 A2 B37 B21 DR4
11	5	85 ×10 /l	A2 A29 B12 B40 DR4 DR7
12	4	46 ×10 /l	A2 B12 B15 DR4
13	4	69 ×10 /l	A2 A11 B12 B27 DR4 DR5
14	3	55 ×10 /l	A1 A10 B17 B21 DR4
15	6	87 ×10 /l	A2 A3 B12 B35 DR1 DR4
16	20	85 ×10 /l	A2 A31 B12 B16 DR2 DR4
17	3	76 ×10⁹/l	A1 A31 B35 DR1 DR4
18	AuIG 2 weeks	LP 0 4×10 /l	A1 A3 B17 B35 DR3 DR5
	DP 3	1 3×10 /l	
19	AuTG 3 months	2 0×10 /l	A2 A30 B8 B40 DR3 DR4
	DP 4	TP 55 ×10 /l	
20	AuTG 2	95 ×10 /l	A2 A32 B5 B8 DR1 DR3
		TP 2 0×10⁹/l	
21	2 weeks	1 5×10⁹/l	A1 A10 B8 DR4
		TP 90 ×10⁹/l	

[1]AuTG = Aurothioglucose [2]DP = D penicillamine [3]TP = Thrombocytopenia [4]LP = Leucopenia

Definition of drug toxicity and clinical course Monitoring for toxicity included counting of leucocytes and platelets once a week during the first months thereafter the interval was gradually prolonged Leucopenia and thrombocytopenia were considered drug-induced if counts in venous blood samples fell below 2 0 $\times 10^9$/l or 120 $\times 10^9$/l, respectively on at least two separate consecutive dates and restoration of counts was observed after stopping the drug

Aurothioglucose (50 mg i m) was given weekly up to 1000 mg and then tapered off D penicillamine was prescribed in initial daily doses of 250 mg, increments were made up to 750 depending on the clinical response

Sixteen patients were symptomless and were managed by stopping the drug and close observation as out patients The remaining 5 patients had either severe thrombocytopenia with bleeding (patients 1, 2 and 5), agranulocytosis (patient 18) or fever (patient 6), all on AuTG

Control groups There were 2 patient control groups, one for each treatment regimen The first control group comprised 65 RA patients having received aurothioglucose (AuTG controls) The second patient control group consisted of 24 patients who were part of the AuTG control group but who had received D-penicillamine as well (DP controls) In these patient groups drug toxicity had not occurred during uninterrupted treatment courses of minimally one year The third control group was a panel of 277 blood donor volunteers (healthy controls)

Statistical methods Significance of the associations in the two-by-two tables was tested by Fisher's exact test without correction for the number of antigens The p-values are reported with the Tables We refrained from calculating relative risks since several tabular entries were zero, which makes the calculation impossible unless assumptions are made

HLA typing Typing for HLA-A, B, C and DR antigens was done with standard NIH-microlymphocytotoxicity techniques HLA-DR typing was done twice with two different sets of reagents on enriched B-lymphocyte suspensions obtained after rosetting with AET-treated sheep red blood cells Sera for the detection of DR1-DR10 were partly of local origin, all sera being standardized against the 8th International Histocompatibility Workshop sera Family studies were not performed for confirmation of homozygosity if only a single DR antigen was detectable

Results

The HLA phenotype frequencies in RA patients with haematotoxic side effects on AuTG and DP are shown in Table 2 Compared to RA controls an increased frequency was observed only for antigens B8 and DR3 Although antigen DR4 was high in frequency, the difference compared to RA controls was not significant The DR2 antigen frequency

Table 2
HLA phenotype frequencies in RA patients with haematotoxic reactions (RAT) RA controls (RAC) and healthy controls (HC)

Antigen	Haemato toxic (N = 21)	RA controls (N = 65)	Healthy controls (N = 277)
B8 +ve	12 (58%)	16 (25%)	62 (22%)
DR3 +ve	9 (42%)	11 (15%)	64 (23%)
DR2 +ve	2 (10%)	12 (18%)	89 (32%)
DR4 +ve	14 (68%)	36 (55%)	54 (20%)
	RAT *vs* RAC	RAT *vs* HC	RAC *vs* HC
B8 +ve	p <0 01	p <0 001	NS
DR3 +ve	p <0 02	p = 0 04	NS
DR2 +ve	NS	p = 0 03	p = 0 03
DR4 +ve	NS	p <0 001	p <0 001

Table 3
HLA phenotype frequencies in RA patients with haematotoxic reactions: association with type of adverse reaction

Phenotype	Leucopenias (LP) (N = 6)	Thrombocytopenias (TP) (N = 19)	RA controls (RAC) (N = 65)
B8 +ve	4 (67%)	11 (58%)	16 (25%)
DR3 +ve	4 (67%)	8 (42%)	11 (17%)
B8 and/or DR3 +ve	6 (100%)	11 (58%)	20 (31%)
DR2 +ve	0 (0%)	2 (11%)	12 (18%)
DR4 +ve	2 (31%)	14 (74%)	36 (55%)
	LP vs RAC	TP vs RAC	
B8 +ve	NS	p <0.02	
DR3 +ve	p = 0.02	p = 0.05	
B8 and/or DR3 +ve	p = 0.001	p = 0.03	
DR2 +ve	NS	NS	
DR4 +ve	NS	NS	

Table 4
Drug dependency of HLA associations with haematotoxicity: aurothioglucose (AuTG) and D-penicillamine (DP)

Phenotype	Aurothioglucose (AuTG) Toxic (N = 9)		AuTG controls (N = 65)	D-penicillamine (DP) Toxic (N = 14)		DP controls (N = 24)
B8 +ve	8 (88%)	p <0.001	16 (25%)	5 (36%)	NS	4 (17%)
DR3 +ve	8 (88%)	p <0.001	11 (17%)	3 (21%)	NS	3 (13%)
B8 and/or DR3 +ve	9 (100%)	p <0.001	20 (31%)	6 (43%)	NS	4 (17%)
DR2 +ve	1 (11%)	NS	12 (18%)	1 (7%)	NS	4 (17%)
DR4 +ve	3 (33%)	NS	36 (55%)	12 (93%)	NS	17 (71%)

* This group comprises 12 thrombocytopenias all in DR4 positive patients. NS = not significant

was lower in both toxic and non-toxic RA patients when compared to healthy controls.

With reference to type of reaction (Table 3) it appeared that all 6 leucopenias (4 on AuTG, 2 on DP) occurred in B8- and/or DR3-positive patients (p = 0.001). These antigens were also increased in the 19 patients with thrombocytopenia (7 on AuTG, 12 on DP) (p = 0.03). When compared to RA-controls, the frequencies for DR2 and DR4 were not significantly different from those found in the control group.

Upon analysis for type of exposure (reaction to AuTG or DP, Table 4), it was observed that the association between AuTG-induced reactions and antigens B8 and/or DR3 was complete (p <0.001). From these 9 B8 and/or DR3 positive patients, 7 (78%) possessed both antigens, as compared to only 7 out of 20 (35%) B8 and/or DR3 positive RA control

patients Looking at the DP-induced reactions, the frequencies of B8 and DR3 antigens were not significantly increased There was a positive correlation between DR4 and DP-induced haematotoxicity It appeared that all 12 patients with DP-induced thrombocytopenia were DR4 positive

Thus, haematotoxicity on AuTG seems to be primarily associated with B8 and/or DR3, while thrombocytopenia on DP is associated with DR4

Discussion

The present data indicate that certain HLA phenotypes are risk factors for the development of haematotoxic reactions to aurothioglucose (AuTG) and D-penicillamine (DP) in patients with rheumatoid arthritis (RA) All patients with AuTG-induced haematotoxicities were B8- and/or DR3-positive, whereas DR4 was present in all cases of DP-induced thrombocytopenia

These observations indicate a genetic predisposition in RA patients to develop haematotoxic reactions to AuTG and DP Furthermore, this genetic predisposition may be different for AuTG- and DP-induced reactions

In the patients with AuTG-induced reactions the strength of correlations with either B8 or DR3 was equal In view of the linkage disequilibrium that exists between these antigens, the high combined occurrence in these patients suggests that AuTG induced haematotoxicity is primarily related to the haplotype containing both alleles. The fact that these phenotypes were found in patients with thrombocytopenia as well as leucopenia on AuTG raises the possibility that patients with this phenotype are at increased risk for both types of haematotoxicity. Coblin and coworkers (Coblin et al 1981) observed an excess of HLA-DR3 in 15 RA patients with severe aurothiomalate-induced thrombocytopenia, 12 being positive for this antigen They did not mention the frequency of the antigen B8 Our results on AuTG are in agreement with theirs on aurothiomalate, with the addition that the correlation with B8 was equally strong.

There seems to be less conformity on gold salt-induced leucopenia since, in contrast with our results, Gran and co-workers (1983) were unable to demonstrate a correlation with HLA antigens in RA patients with aurothiomalate-induced leucopenias It is not inconceivable that differences in inclusion criteria contribute to this discrepancy By rigorous inclusion criteria we have tried to avoid including possible spontaneous biological variation in blood cell counts in the side effect group This will increase the contrast between the patients with and without side effects

It is not yet clear in which way HLA antigens participate in adverse reactions. In non-RA patients with drug-induced thrombocytopenia on exposure to various drugs, there seems to be no correlation with HLA-B antigens (Goebel et al 1976) In RA patients the possibility remains that HLA-B8-DR3 is involved in the more severe reactions to antirheumatic drugs This is demonstrated by another toxic reaction that sometimes complicates treatment with gold salts and DP, i e. proteinuria In a survey of 44 proteinuric RA patients we found the nephrotic syndrome, as compared to symptomless proteinuria, preferentially in patients with HLA-DR3 (Speerstra et al 1983) From our data it cannot be excluded that B8-DR3 positives are more prone to severe haematotoxic reactions at least on exposure to AuTG, since the majority required hospitalization or supportive treatment In contrast with the AuTG group, this phenotype seems to play no important role in the production of haematological side effects of DP So far as the severity of the reactions is concerned, there were no differences between the patients with and those without B8 and

DR3 antigens. However, a high prevalence of DR4 was found in this group. All patients with DP-induced thrombocytopenia were DR4 positive, of whom 25% were DR4-DR blank, the latter frequency being almost twice as high as that in the corresponding controls. Although the prevalence of DR4 was high in the controls as well, this contrast was statistically significant, indicating that patients with phenotype DR4 are at increased risk of developing thrombocytopenia on DP. This possibility has been proposed earlier by Dawkins and co-workers (Dawkins et al. 1983).

It is conceivable that other as yet unexplored factors contribute to an increased risk as well. This possibility was demonstrated by Batchelor et al. (1980) in hypertensive patients with hydralazine-induced SLE: in addition to HLA-DR4, the risk of having this complication was increased up to 100% if the patient was a female, over 40 years old and a slow acetylator. With reference to drug toxicity in RA, other factors such as low titres of IgA (van Riel et al.1984) and specific gold antibodies of the IgE class (Bretza et al. 1983) have been reported in connection with skin rashes. In these studies HLA antigens were not investigated. The present data on correlations between HLA antigens and haematotoxic reactions induced by AuTG or DP await confirmation. In the meantime, we believe it possible that RA patients at increased risk for haematotoxic reactions can be defined by tissue typing. This is so because the associations with HLA phenotypes in the group with AuTG-, as well as DP-induced reactions were complete. The major goal – to try to avoid major complications – could be achieved better by means of intensified monitoring and adaption of dosage schedules in the patients at risk while, on the other hand, in the low risk patient group the frequency of these tests could be minimalized. This would contribute to a less uniform and cost-saving approach in antirheumatic treatment (Liang & Fries 1976).

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CHAPTER 5

THE INFLUENCE OF HLA PHENOTYPES ON THE RESPONSE TO PARENTERAL GOLD IN RHEUMATOID ARTHRITIS

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SUMMARY

One-hundred and ten patients with rheumatoid arthritis (RA) were studied for a possible influence of HLA phenotypes on the reaction to parenteral gold in the first 6 months of treatment, in terms of both clinical response and toxicity. Frequencies of HLA B8 and DR3 were significantly increased in patients who responded excellently to gold treatment as compared with non-responders ($p = 0.04$ for both antigens). On the other hand, HLA DR7 was increased in non-responders versus excellent and moderate responders ($p < 0.03$). Drug toxicity was higher in excellent than in non-responders ($p = 0.04$), being exceptionally high in male excellent responders (85% versus 33% in females , $p < 0.01$), probably due to the increased frequency in B8 and DR3 in the excellent responder group as a whole and in the excellent responder males in particular.

We conclude that HLA antigens B8 and DR3 co-determine both toxicity and excellent clinical response to parenteral gold, whereas the presence of DR7 is associated with non-response. In addition, we found sex differences in reaction to parenteral gold, which may be related to an increased frequency of HLA B8 and DR3 in male RA patients.

INTRODUCTION

There is convincing evidence that gold salts are effective in rheumatoid arthritis (RA) (The Cooperating Clinical Committee of the American Rheumatism Association 1973). However, treatment with these drugs is hampered by the frequent occurrence of side effects. In addition, not all RA patients respond favourably to gold therapy. Recent studies indicate a genetic predisposition to the development of gold toxicity, especially proteinuria (Wooley et al. 1980) and thrombocytopenia (Coblyn et al. 1981,Speerstra et al. 1985). At present there is only scanty information on whether there is also a genetic predisposition to a favourable response to gold therapy. Previously we have reported an association between HLA DR3 and excellent clinical response, but numbers in that study were small (Van Riel et al. 1983). The present study aimed to extend these observations; therefore all patients receiving aurothioglucose in the past three years under the standardized conditions proposed by Gottlieb (Gottlieb 1979) were studied.
On the basis of the present results, it seems that HLA B8 and HLA DR3 positivity plays a role in drug response, Patients with these particular HLA phenotypes had a greater chance of a favourable outcome, but also an increased risk of toxicity in the first 6 months of treatment. On the other hand, antigen DR7 seemed to increase the risk of a poor response.

MATERIALS AND METHODS

Selection of patients. Patients included in this study were treated at three departments of rheumatic diseases in the South-Eastern part of The Netherlands. All patients with classical or definite rheumatoid arthritis and treated with aurothioglucose (AuTG) between 1980 and 1984 were included for clinical evaluation. Adequate evaluation was available on 110 patients of this cohort.

Drug prescription and monitoring. After a test dose of 10 mg patients received 50 mg AuTG weekly up to the 20th week, after which the dose was reduced to 50 mg every 2-4 weeks. Patients had a monthly check-up including a questionnaire, physical examination and venipunction for determination of ESR and haemoglobin. In addition, monitoring for haematological and nephrological side effects (leucocyte count and differential, platelet count and routine testing for proteinuria) was initially done weekly and, during maintenance

Table I. Grading of the four components of IDA.

Grade	Duration of morning stiffness (min)	Observer assessment	Hb(mmol/l) male	Hb(mmol/l) female	ESR(mm/h)
1	<10	very good	>8.7	>7.4	0-20
2	10-30	fair	8.1-8.6	6.9-7.3	21-45
3	31-120	poor	6.2-8.0	5.3-6.8	46-80
4	>120	very poor	<6.1	<5.2	>81

therapy, at least once every 4 to 6 weeks. Patients were considered to have developed an adverse reaction to AuTG if any of the following signs were observed: severe pruritis, rash or stomatitis, which diminished after stopping treatment; proteinuria exceeding 500 mg/24 h for more than two weeks; a fall in platelet count below 120.000/mm^3; white blood cell count less than 2000/mm^3 or an absolute polymorphonuclear count below 1500/mm^3.

Evaluation of drug response. A modification of a recently published scoring system for patients with RA was used to evaluate response (Van Riel et al. 1983). The four parameters of the Index of Disease Activity (IDA) and its gradings are shown in Table 1. The degree of improvement upon therapy in each patient was established by determining the percentage of improvement from baseline (PIDA). Patients were considered to have shown an excellent response if this IDA at month 6 was less than or equal to 1.25 and the PIDA was at least 40%. Non-responders were patients who either improved less than 10% or deteriorated. Moderate responders were patients not covered by the previous classifications.

HLA typing. HLA typing was done with standard NIH microlymphocytotoxicity techniques for HLA ABC and DR antigens. DR typing was done twice with two different sets of reagents on enriched B lymphocyte suspensions obtained after rosetting with AET treated sheep red blood cells. Sera were partly of local origin, all sera being standardized against the 8th and 9th International Histocompatibility Workshop sera.

Statistical analysis. Fisher's exact test and the chi square test were used for comparison of two proportions to assess statistical significance of data. P values were not multiplied by the number of antigens tested for.

RESULTS

A total of 110 patients were evaluated. Ninety-one (83%) improved, of whom 34 (31%) responded excellently and 57 (52%) moderately. The remaining 19 (17%) showed no significant improvement or aggravation of disease symptoms. In accordance with the literature (6), the HLA-DR4 frequency was found to be significantly increased (57%) and the HLA-DR2 frequency significantly decreased (18%) in the total patient group as compared with the healthy control group ($p < 0.001$ and $p = 0.01$ respectively). No statistically significant differences were found between the total patient group and the group of healthy controls as regards the distribution of the other HLA antigens.

Table II. Frequency of HLA antigens and response to aurothioglucose

HLA	Excellent responders n = 34	Moderate responders n = 57	Non responders n = 19	Controls n = 277
B8	13 (38%)*	9 (16%)	2 (11%)	64 (23%)
DR1	12 (35%)	17 (30%)	4 (21%)	70 (25%)
DR2	6 (18%)	10 (18%)	4 (21%)	89 (32%)
DR3	12 (35%)*	9 (16%)	2 (11%)	64 (23%)
DR4	18 (53%)	36 (63%)	9 (47%)	54 (20%)
DR5	3 (9%)	6 (11%)	3 (16%)	65 (24%)
DRw6	2 (6%)	6 (11%)	3 (16%)	73 (26%)
DR7	4 (12%)	4 (7%)	7 (37%)**	56 (20%)
DRw8	1 (3%)	2 (4%)	1 (5%)	16 (6%)
DRw9	0 -	4 (7%)	1 (5%)	8 (3%)
DRw10	2 (6%)	2 (4%)	3 (16%)	2 (1%)

* $p = 0.04$, excellent versus no response.
** $p < 0.03$, no response versus excellent and moderate response.

HLA antigens and drug response (table II).

The frequencies of antigens B8 and DR3 were highest in the excellent responder group and lowest in the non-responder group. These differences were statistically significant ($p = 0.04$). At the same time the frequency of antigen DR7 was significantly decreased in the excellent responder group as well as in the moderate responder group as compared with the non-responder group (p 0.03).

HLA-phenotype, sex, drug response and toxicity (Table III and Figure 1).

Fourty-four patients developed toxicities, mainly skin rashes ($n = 34$). Of the 44 toxic patients 14 were HLA B8 positive (32% versus 15% in non-toxic patients, n.s.) and 14 were DR3 positive (32% versus 14% in non-toxic patients, $p = 0.04$). Patients with skin rashes showed similar differences for B8 (38% versus 9%, $p = 0.01$) and DR3 (29% versus 14%, n.s.). The incidence of toxic reactions was highest in the excellent responder group and lowest in the non-responder group (53% and 26% respectively; $p = 0.04$). Toxicity was exceptionally high in excellently responding males (85%). The difference from females who reacted excellently was statistically highly significant ($p < 0.01$).

No significant differences in toxicity existed between both sexes in the moderate and the non-responder group.

Table III. Side effects and response to aurothioglucose.

	side effects		
response category	female patients	male patients	total
Excellent responders (n=34)	7/21 (33%)	11/13 (85%)*	18/34 (53%)
Moderate responders (n=57)	14/37 (38%)	7/20 (35%)	21/57 (37%)
Non-responders (n=19)	3/13 (23%)	2/6 (33%)	5/19 (26%)

$p < 0.01$ (male compared with female)

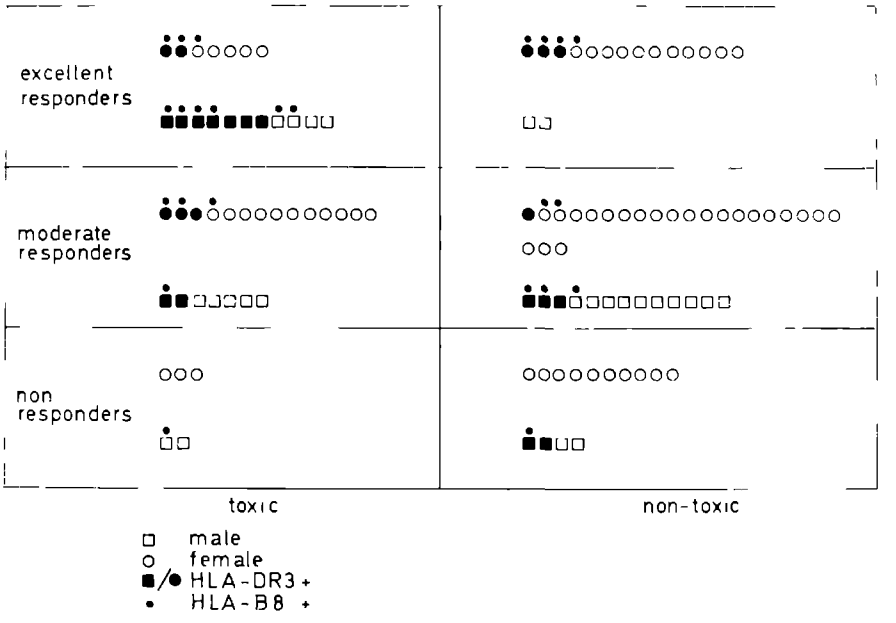

Figure 1. Data about toxicity, degree of response, HLA-DR3 and B8 positivity in 110 aurothioglucose treated patients. Each symbol represents a patients.

As to type of response, no statistically significant difference was found between the sexes. There was, however, a sex difference in HLA B8 and DR3: from the total of 39 males, 12 (31%) were B8 positive as compared with 12 out of 71 female patients (17%; n.s.); for DR3 the figures were 14 (30%) and 9 (13%) ($p < 0.01$).

DISCUSSION

The present data indicate that in patients with active rheumatoid arthritis the reaction to parenteral gold therapy, in terms of both toxicity and effectiveness, is co-determined by HLA phenotypes. As for effectiveness, excellent responderships was found to be associated with HLA B8 and DR3, whereas poor responderships was associated with DR7. The high coincidence of toxicity and excellent responderships found in this study, suggests that there is a subgroup of RA patients who have increased reactivity to gold treatment and,

furthermore, that these patients are more frequently B8 and/or DR3 positive. The fact that the group of excellently responding male patients showed an exceptionally high incidence of toxicity is most probably related to the higher prevalence of the latter antigens in this patient catagory. In any case, it cannot be concluded from these data that male sex per se represents an independent factor determining reaction to gold therapy in patients with rheumatoid arthritis.

It is important to realize that our results concern drug effects during the first 6 months of treatment and that the conclusions do not necessarily apply to late drug manifestations. This might explain, at least in part, the discrepancy between studies of drug toxicity and HLA phenotypes. Correlations with antigens B8 and DR3 are probably primarily restricted to drug effects occurring in the early phase of treatment; when late drug effects are included, these correlations may disappear. Support for this is found in a study on drug-induced proteinuria previously reported by us (Speerstra et al. 1983). In this study we found a significant correlation between early onset proteinuria and HLA DR3. This correlation was lost, however, when late onset proteinuria was included. The same may be true for dermatitis, the predominant side effect in this study, which in our patients proved to be associated with HLA DR3, in contrast to other reports (Dequeker et al. 1984, Panayi et al. 1978).

Only two reports have so far studied the effectiveness of gold treatment in relation to HLA phenotypes. In the study of Van Riel et al (Van Riel et al. 1983), a positive correlation was found between a favourable response and DR3 positivity. Recently this observation has been confirmed by Bensen et al (Bensen et al. 1984). However, O'Duffy et al could not confirm this observation (O'Duffy et al. 1984). They reported a relationship between the absence of antigen A3 and the simultaneous presence of antigen DR4 and poor repondership on gold. In our study, the frequency of these particular antigens was not different in the various responder categories.

To our knowledge this is the first report on sex differences in drug response in patients with rheumatoid arthritis. The higher prevalence of antigen DR3 observed in the male patient category indicates that the genetic background differs from that of female patients leaving the possibility that drug response and probably also certain disease manifestations may show differences between both sexes. A recent study has also concentrated on this aspect. Dequeker et al (Dequeker et al. 1984) reported an increased frequency of antigen DR4 in male patients who had developed RA before age 40. In his study,

however, the development of toxic reactions to gold and d-penicillamine was not essentially different in males as compared withfemales.

In conclusion, our data provide evidence that HLA antigens play a role in the reaction of patients with rheumatoid arthritis to parenteral gold therapy, not only in terms of toxicity, but also in terms of effectiveness of the drug, both being associated with HLA B8 and DR3. In addition, we found that these antigens are more frequently found in male RA patients, which might explain the different frequency of toxicity found in our study. These observations, however, need further confirmation by other studies.

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CHAPTER 6

REMARKABLY SIMILAR RESPONSE TO GOLD THERAPY IN HLA IDENTICAL SIBS WITH RHEUMATOID ARTHRITIS

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Two pairs of sibs with definite rheumatoid arthritis responded remarkably similar to parenteral gold therapy, both in terms of toxicity and efficacy Both pairs proved to be HLA identical. One of the pairs possessed the HLA antigens B8 and DR3, that have been associated with both drug toxicity and excellent clinical response. The other pair did not posses one of these antigens, suggesting that the reaction to gold therapy in patients with rheumatoid arthritis may be determined by other HLA and/or genetical factors coded for by chromosome 6.

INTRODUCTION

Paranteral gold still is a cornerstone of drug treatment of rheumatoid arthritis (RA). Disadvantages of the drug are its frequent toxicity and its ineffectiveness in some patients. Recently, associations with certain HLA antigens have indicated that genetic factors play a role in both gold toxicity and effectiveness (1-4). HLA antigens involved include HLA B8 and DR3 in gold toxicity and DR3 in excellent respondership to the drug (1,2,3). Another study (4) found a relationship between the absence of antigen A3 and the simultaneous presence of antigen DR4 and bad respondership to parenteral gold.
In the present report we describe two pairs of sibs with rheumatoid arthritis who responded remarkably similar to parenteral gold therapy, both in terms of toxicity and clinical response, one of the sib pairs doing so during two subsequent courses of parenteral gold. Tissue typing showed that both pairs of sibs were HLA identical; whereas one pair of sibs was HLA B8 and DR3 positive, the other one did not possess HLA antigens known to be associated with toxicity of clinical response. This observation suggests that other HLA antigens and/or genetic factors coded for by chromosome 6 play a role in the reaction to parenteral gold and patients with rheumatoid arthritis.

METHODS

In the context of a large study on families with multiple cases of rheumatoid arthritis, we detected two sib pairs with definite rheumatoid arthritis according to the revised ARA diagnostic criteria (5), who responded remarkably similar to parenteral gold therapy, i.e. aurothioglucose. Toxicity was monitored by regular questionaire, physical examination, blood counts and urinalysis. Response to gold therapy was evaluated according to a modification of a recently published scoring system for patients with rheumatoid arthritis (3,6). Parameters used in this evaluation are the duration of morning stiffness, observer assessment, hemoglobin and ESR. HLA typing was done with standard NIH microlymphocytotoxicity techniques for HLA ABC and DR antigens. Sera were partly of local origin, all sera being standardized against the 8th and 9th International Histocompatibility Workshop sera.

Table II. Reaction to parenteral gold

	sib pair I		sib pair II[1]		sib pair II[2]	
patient	A	B	C	D	C	D
toxicity type	dermatitis	dermatitis	dermatitis stomatitis	dermatitis	dermatitis	dermatitis
after	5 wk	3 mo	4 mo	8 mo	5 mo	9 mo
at dose (mg)	250	850	1200	1100	500	800
response type	excellent	excellent	moderate	moderate	moderate	moderate
after	2 mo	3 mo	3-4 mo	4 mo	6 mo	4 mo
Hb (mmol/l)7.1						
a[4]	8.4	7.3	8.8	7.5	7.9	7.1
b[5]	8.1	7.5	8.1	6.5	8.1	7.1
ESR (mm/h)25						
a	36	66	33	30	59	25
b	19	21	10	15	28	13
MS[3] (min)15'						
a	120'	120'	120'	120'	15'	15'
b	15'	0'	15'	10'	0'	0'
doct.assessment	excellent	excellent	excellent	excellent	good	excellent

[1] First treatment episode
[2] Second treatment episode
[3] MS = morning stiffness
[4] a = at the onset of therapy
[5] b = at evaluation of response

Table I. Patient Characteristics

	sib pair I		sib pair II	
	pat A	pat B	pat C	pat D
sex	F	F	M	F
age at onset RA	53	54	53	72
joint erosions	±	+	+	+
nodules	-	-	-	-
serology				
Rose/Waaler	1/64	1/128	-	1/256
latex fixation	-	nd	-	+
ANA	-	-	-	+
HLA haplotypes	A10Bw21DR3/A3B40DR4		A2B7DR4/A2B18DR4	

SIB PAIRS

All four patients had symmetrical polyarthritis. Patient characteristics and data on the reaction to parenteral gold are represented in table I and II respectively.

Sib pair 1 (two sisters). Patient A got rheumatoid arthritis at the age of 53. There was no sufficient response to NSAID. At the age of 54 treatment with aurothioglucose was started. After only 250 mg of parenteral gold, she developed a skin rash predominantly round the shoulder and the elbow, necessitating to stop the treatment. At the same time there were only minimal signs of arhritis. The skin rash disappeared in the month thereafter. Patient B, her elder sister, developed rheumatoid arthritis at the age of 54. Treatment with NSAID and hydrochloroquine was not persistently successful. At the age of 58 treatment with aurothioglucose was instituted. After 850 mg of the drug,given in a period of three months, she developed a macular papillar rash, for which treatment had to be stopped. Thereafter, the skin rash gradually disappeared. At the time of the skin rash, there was no disease activity at all.

Sib pair 2 (brother and sister). Patient C got rheumatoid arthritis at the age of 53. Initially, he had been treated with NSAID and hydroxychloroquine,

which was initially effective but later on not sufficient to control disease activity. At the age of 58 years, gold therapy was instituted. After 4 months he developed dermatitis at the right elbow, the nates and in the groins. There was a slight stomatitis. Gold was stopped. At that time there was no disease activity anymore. Approximately five and a half years later, gold was reinstituted because of a disease flare. This treatment had to be stopped after a cumulative dose of 500 mg, because of skin rashes at the ear,wrist and-scrotal area. Clinical response was good, although some rest swelling of the joints was present. Patient D, his elder sister, got rheumatoid arthritis at the age of 71. She was initially treated with NSAIDs and hydroxychloroquine, without clinical effect. At the age of 72, gold therapy was started; after four months of treatment, there were no signs of active arthritis anymore. At the cumulative dose of 1100 mg she got a moderately severe dermatitis of the abdominal region and the back. Gold was stopped. There were no signs of disease activity at that time. Because of a flare, gold was reinstituted one and a half year later. After three months of therapy, there were no signs of disease activity anymore. At a total dose of 800 mg (after 9 months) she developed a dermatitis, predominantly localized at the arms and trunk, necessitating to stop the treatment. At that time there was only slight disease activity.

DISCUSSION

This report shows a remarkably identical reaction to parenteral gold therapy in two sib pairs, both in terms of toxicity and favourable clinical response to the drug. Tissue typing showed that the sib pairs were HLA identical.
Toxicity on parenteral gold therapy has been found in some studies to be associated with certain HLA antigens, namely HLA B8 and DR3 (2,7,8,9,10). These associations were predominantly found for gold-induced proteinuria (7,8) and thrombocytopenia (9,10). Interestingly, an association between the most frequent side effect i.e. skin rash and HLA antigens has been found in only a few studies (2,11,12). Bardin et al (11) found an association with the haplotype A1 Cw7 B8 DR3 and our group has recently reported an association with HLA B8 and DR3 (12). It should be stressed, however, that other groups have been unable to find associations between HLA antigens and gold toxicity (13,-14).
Interestingly, the patients in the two sibships developed the same kind of

toxicity and in addition, showed the same favourable clinical response to the drug. One of the sib pairs was positive for HLA B8 and DR3, antigens, that have been associated with both gold toxicity (1,2,7,8,9,10,11) and excellent clinical response (3,12). The other sib pair, however, did not possess these antigens. Both patients of the latter sib pair received two courses of gold and at both times reacted identical to this treatment. This observation suggests that other genetic factors, be it HLA and/or other genetic factors associated with chromosome 6, play a role in the reaction to parenteral gold therapy.

Although suggestive, the present data of course are essentially anecdotal and need to be confirmed by more extensive studies. There is, however, accumulating evidence that genetic factors play a role in the way patients react to antirheumatic drugs, both in terms of side effects and efficacy. It may therefore be important in case of multiple case families to take a history of drug toxicity and clinical response. If HLA identity proves to be of any importance, HLA typing in such cases might become important in the future, especially in case of severe side effects.

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CHAPTER 7

COMPARISON OF THE EFFECT OF ANTIRHEUMATIC DRUGS IN FIRST DEGREE RELATIVES WITH RHEUMATOID ARTHRITIS

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We selected 13 pairs of first degree relatives with definite or classical rheumatoid arthritis, who had undergone treatment with identical second line drugs (parenteral gold, d-penicillamine), to look for possible concordances in drug effects, both in terms of toxicity and efficacy. The selected 13 pairs, had 15 paired therapeutic episodes. Ten out of 15 paired therapeutic episodes were concordant for toxicity (expected 7.5); patients with a toxic relative developed more frequently drug toxicity than patients with non-toxic relatives (relative risk 1.8, p = 0.146). However, favourable respondership in a relative had no predictive value. Numbers in the 3 haplotype sharing categories were too small to detect any influence of the degree of haplotype sharing on concordance. The only two HLA identical pairs were remarkably similar in reaction to parenteral gold, both in terms of toxicity and efficacy. Studying the influence of sex on concordance showed that 4 out of 5 concordances for toxicity were in sexidentical pairs, whereas 4 out of 5 concordances for non-toxicity were in sex non-identical pairs. Our data suggest that parenteral gold- or d-penicillamine-induced toxicity in a first degree relative may be a risk factor for patients with RA to be treated with these drugs.

INTRODUCTION

Genetic factors play not only a role in the pathogenesis of rheumatoid arthritis (RA), but also seem to be involved in the way patients with RA react to so-called second-line drugs (1-8). These studies have been carried out in groups of unrelated patients with RA and have indicated associations of certain HLA antigens with drug toxicity in d-penicillamine (1,2,4,5,8) and with both drug toxicity (1-8) and efficacy (6,7) in parenteral gold treatment. No studies have been performed in relatives with RA, to look for possible similarities in drug response, apparently because such a study has to deal with many obstacles. However, this type of information would be useful, since patients frequently want to be informed on the chance to have a similar reaction to a certain type of treatment as a known relative. This is especially true, in case of severe drug toxicity in the latter. In the present study we report on pairs of first degree relatives with RA, obtained from a large study of families with multiple cases of RA, who had been treated with the same second line drug, either parenteral gold (aurothioglucose, AuTG) or d-penicillamine (DP). We looked for concordance in both (non)toxicity and (non)efficacy and for the possible influence of HLA haplotype sharing and sex identity.

PATIENTS AND METHODS

Patients. We selected one pair of patients from each multiple case family, using the following criteria:

- both patients had to have definite or classical rheumatoid arthritis (9)
- they had to have been treated with the same second-line drug
- they had to be first degree relatives (sib-sib, parent-child)
- availability of adequate clinical and drug therapy data.

In case more than two cases per family were eligible, we selected pairs of the youngest and the eldest ones in terms of start of treatment. Selection was done without knowledge of the reaction to therapy and without knowing the results of HLA studies. In addition, sex was not a selection criterium. Using the above procedure, we selected 13 pairs of RA patients. Two of these pairs had been treated with both parenteral gold and d-penicillamine. This resulted in 15 paired therapeutic episodes to be studied. Of the 26 patients, 15 were females. Of the 13 pairs, 9 were sib-sib - and 4 parent-child pairs.

Drugs, dosage schedules, and toxicity monitoring. After a test dose of 10 mg patients received 50 mg AuTG weekly up to the 20th week, after which the dose was reduced to 50 mg every 2-4 weeks. D-penicillamine was given in one 250 mg tablet daily, and the dosage slowly raised to a maximum of 750 mg per day depending on the clinical response. Patients had a monthly check up, including a questionaire, physical examination and venapunction for determination of sedimentation rate and hemoglobin. In addition, monitoring for haematological and nephrological side effects (leukocyte count and differential, platelet count and routine testing for proteinuria) was initially done weekly and, during maintenance therapy, at least once per 4 to 6 weeks. Patients were considered to have developed an adverse reaction to AuTG or DP if any of the following signs were observed: severe pruritis, rash or stomatitis, which diminished after stopping treatment; proteinuria exceeding 500 mg/24 h for more than two weeks; a fall in platelet count below 120.000/mm^3; white blood cell count less than 2000/mm^3 or an absolute polymorphonuclear count below 1500/mm^3.

Evaluation of drug response. A modification of a recently published scoring system for patients with RA (6,10) was used for evaluation of response. The four parameters of the index of Disease Activity (IDA) and its gradings are shown in Table I. The degree of improvement on therapy in each patient was obtained by determining the percentage of improvement from baseline. Patients were considered to have responded if they showed an improvement of at least 20% within 6 months treatment. Non-responders were patients who either improved less than 20% or deteriorated.

Table I. Grading of the four components of IDA.

Grade	Duration of morning stiffness (min)	Observer assessment	Hb(mmol/l) male	Hb(mmol/l) female	ESR(mm/h)
1	<10	very good	>8.7	>7.4	0-20
2	10-30	fair	8.1-8.6	6.9-7.3	21-45
3	31-120	poor	6.2-8.0	5.3-6.8	46-80
4	>120	very poor	<6.1	<5.2	>81

Statistical analysis. The expected number of pairs in which both members reacted in a similar way (either in regard to (non)toxicity or clinical response) was calculated under the assumption of independence of these episodes. Chi-square statistics were applied to the two by two tables and two-sided p values were calulated.

RESULTS

Data on the 26 patients (13 pairs) and their 30 therapy episodes are given in table II. Of the 30 therapy episodes, 22 were on aurothioglucose, and 8 on d-penicillamine. Of the 30 therapy episodes, 15 (50%) were associated with drug toxicity at some time during treatment, a value somewhat higher than that in populations of unrelated patients, i.e. 30 to 40 percent (11,12). Twenty-two of the 30 episodes could be classified as a (favourable) response to drug treatment. If the 30 therapeutic episodes had been in non-relatives, the following calculations can be made for the expected number of concordant pairs. Since 15 of the 30 patients developed toxicity, i.e. a chance of 0.5, the chance of 2 patients to be concordant for toxicity is 0.5 x 0.5 = 0.25. Similarly the chance of concordance for non-toxicity is 0.5 x 0.5 = 0.25. Therefore the total chance for concordance of (non)toxicity is 0.25 + 0.25 = 0.5; this means an expected number of 0.5 x 15 pairs = 7.5 concordant pairs. Likewise calculations can be made for the chance of concordance for response (= 0.5377) and for non-response (= 0.0711), leading to a total chance of concordance for (non)response of 0.5377 + 0.071 = 0.6088; this means an expected number of 0.6088 x 15 pairs = 9.132 concordant pairswith respect to (non)response.

Toxicity. Table III represents the toxicities divided into those in the youngest and those in the eldest ones in terms of sequence of treatment. It can be seen from this table that of the 6 younger patients with an elder first degree relative with toxicity, 5 developed also toxicity, whereas only 4 out of 9 without a toxic relative did so. So having a toxic first degree relative seems to be associated with a risk for toxicity of: 5:6/4:9 = 1.8 (chi square 2.117, p = 0.146).

TABLE II. Clinical response and toxicity in 13 pairs of first degree relatives treated with the same antirheumatic drug, i.e. either aurothioglucose (AuTG) or d-penicillamine (DP)

family, sex, relationship	age at onset of treatment	drug	response[1])	toxicity	HLA haplotypes
I vd Wi					
1 m son	27	AuTG	-	-	A1B8DR5/AW33B7DRw8
2 f mother	67	AuTG	+	dermatitis, alopecia,fever	A1B8DR5/A9B37DRw10
II vd B					
1 f sib	58	AuTG	+	dermatitis	A10Bw21DR3/A3B40DR4
2 f sib	54	AuTG	+	dermatitis	identical
III H					
1 m sib	50	AuTG	+	-	A1B8DR1/A11B27DR1
2 f sib	60	AuTG	+	-	A3B37DRw10/A2B15DR4
IV Key					
1 f sib	74	AuTG	+	dermatitis	A2B7DR4/A2B18DR4
2 m sib	59	AuTG	+	dermatitis	identical
V vd E					
1 f mother	55	AuTG	+	-	A2B15DRw6/A1B27DR1
2 f daughter	31	AuTG	-	polyneuropathy	A2B15DRw6/A1B8DR3
VI Lo					
1 f mother	43	AuTG	+	dermatitis fever	Aw31B5DR4/A3B18DR3
2 f daughter	36	AuTG	+	dermatitis	Aw31B5DR4/A9B37DR4
VII Waa					
1 m father	53	AuTG	+	dermatitis	A3B7DRw6/A10B27DR1
2 m son	27	AuTG	-	diarrhea proteinuria	A2B15DR4/A10B27DR1

VIII	Thie					
	1 f sib	42	AuTG	+	-	A9BxDR2/A2B12DR4
	2 f sib	65	AuTG	+	dermatitis	A3B7DRw10/A2B12DR4
IX	Via					
	1 m sib	37	AuTG	+	dermatitis	A2Bw22DR4/A1B8DR3
	2 m sib	48	AuTg	-	proteinuria	A2Bw22DR4/A2B12DR5
X	Smi					
	1 f sib	(1) 38	AuTG	+	-	A3B15DR1/A28B40DR4
		(2) 51	DP	+	-	
	2 f sib	(1) 39	AuTG	+	dermatitis	A3B15DR1/A3B7DR2
		(2) 51	DP	+	-	A3B15DR1/A3B7DR2
XI	Ko					
	1 m sib	(1) 52	AuTG	-	-	A3B37DRw10/A1B8DR3
		(2) 57	DP	-	-	
	2 f sib	(1) 54	DP	-	-	A3B37DRw10/A2B12DR4
		(2) 56	AuTG	+	-	
XII	Ja					
	1 m sib	36	DP	+	proteinuria thrombocytopenia	A9B15DR4/A1B8DR3
	2 m sib	44	DP	+	-	A1B8DR4/A1B8DR3
XIII	van de V					
	1 f sib	38	DP	+	-	A2B7DR2/A2B7DRw6
	2 m sib	43	DP	-	-	A11B7DR3/A2B7DRw6

1) + = response; - = non-response.

Table III. Distribution of toxicity among youngest and eldest of first-degree relative pairs (see text).

Toxicity in youngest	Toxicity in eldest	
	present	absent
present	5	4
absent	1	5
	6	9

Looking in a somewhat different way to table III, it can be concluded that 5 pairs were concordant for toxicities and 5 for being non-toxic. So we found 10 concordant paired episodes in terms of toxicity or non-toxicity, whereas from the above mentioned calculation, it appears that 7.5 concordant paired episodes would have been expected.

Response. Data in the form of a two by two table are represented in table IV. From this table it can be seen that patients with an elder responder relative, have a chance of 8:12 to react identically themselves, whereas patients with non-response relatives, have a chance of 2:3 to react favourably. The relative risk here is therefore 1.0 (chi square = 0.000). Looking in a different way to table IV, it can be concluded that 9 paired episodes were concordant, 8 for responder and 1 for non-respondership. From the above mentioned considerations, we would have expected a number of 15 x 0.6088 = 9.132 paired concordant episodes. We conclude from these observations that type of response to second line drug treatment in an elder relative is not a predictive factor for a patient to be treated with this drug.

Table IV. Distribution of response among youngest and eldest of a first-degree relative pair (see text).

youngest	eldest	
	response	non-response
response	8	2
non-response	4	1
	12	3

Influence of haplotype sharing and sex. To study the influence of haplotype sharing on concordance, parent-child pairs were left out, leaving 9 sib-sib pairs with the following haplotype status: 2 HLA-identical, 6 haplotype identical and 1 non-identical. These numbers were too small to detect any influence of the degree of haplotype sharing on concordance. The only noticable feature was that the two HLA identical pairs were remarkably similar in both respondership and toxicity.
Studying the influence of sex on concordance showed that 4 out of 5 concordances for toxicity were in sex identical pairs, whereas 4 out of 5 concordances for non-toxicity were in sex non-identical pairs. No special distribution was found for concordances in (non)response.

DISCUSSION

Our data suggest, that first degree relatives with rheumatoid arthritis (RA) treated with similar second line therapy, are more frequently concordant in drug response in terms of toxicity or non-toxicity than would have been expected by chance. In addition, patients with a relative with a history of drug-toxicity, developed toxicity themselves more frequently than patients with non-toxic relatives. In our study favourable response in a relative had no predictive value. Because limitation of numbers, nothing definite could be said on the influence of either haplotype sharing or sex on concordancy.

Studies as the present one may give important information both to the patient and the physician. RA has a tendency to cluster in families (13) and, since the number of so-called second line drugs is limited, there is a considerable chance that two affected members of a multiple case family will be treated in the same way. In clinical practice it is not uncommon for a doctor to be asked by a patient with RA about the chance of developing toxicity on second line drugs, given the fact that a relative has developed a toxicity on a similar drug.

The fact that, to our knowledge no larger studies are available on this subject, is almost certainly due to the obstacles one has to deal with, when facing this problem. Patients have to be collected from different centres, they usually have been treated with different treatment schedules and documentation often is not uniform, to name only a few obstacles.

We were unable to study any influence of the HLA system properly, because of the small number of pairs available. All that could be said is that the only two HLA identical sibs were remarkably similar in their reaction to drug therapy, both in terms of toxicity and clinical efficacy. A detailed clinical description of these patients has been reported elsewhere (14). Studies of groups of unrelated patients with rheumatoid arthritis have indicated associations of particular HLA antigens with both toxicity (1-8) and, recently, clinical efficacy (6-7), indicating that the reaction to second line antirheumatic drugs is influenced by genetical factors. Investigations in families may allow for studying the influence not only of the HLA system, but also of other genetic factors on reaction to drug therapy. Our data suggest that other genetical factors may be present, at least for the development of toxicity. An interesting observation was the distribution of concordances for (non)toxicity over the sex identical and non-identical pairs. Concordances for toxicity seems to cluster in the sex identical groups, whereas concordances for non-toxicity were mainly found in the sex non-identical pairs. Since numbers are small, these observations of course need to be confirmed. Apart from clinical usefulness, family studies on drug reactions may deepen our insight into genetical factors and probably mechanisms operative in reaction to drugs. The present data suggest that this approach may be fruitful and warrant further studies.

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SUMMARY AND CONCLUSIONS

So-called second line antirheumatic drugs, including parenteral gold and d-penicillamine, are still a corner stone in the treatment of the more severe forms of rheumatoid arthritis (RA). The clinical use of these drugs is hampered by the frequent occurrence of side effects. In addition, only part of the patients respond to this form of therapy. The present thesis deals with the possible influence of genetic factors on the reaction to second line antirheumatic drugs, using the HLA system as a genetic marker system. The results of the studies presented in this thesis indicate that certain types of side effects of these drugs are associated with particular HLA phenotypes. In addition, we also found associations with HLA phenotypes and type of response to the drug. As far as side effects were concerned, we studied those toxicities that can be quantified, i.e. proteinuria and hematotoxic side effects due to both parenteral gold and d-penicillamine.

In chapter 2 we report the results of a case control study on a possible association between HLA phenotypes and the development of proteinuria during aurothioglucose (AuTG) or d-penicillamine (DP) treatment in patients with rheumatoid arthritis. Data indicated that HLA-DR3 was markedly increased in the proteinuric patients as compared to non-proteinuric RA controls. In contrast to some studies reported previously, our data showed that HLA-DR3 positive patients were at greater risk during treatment with DP (RR 10.1, $p = 0.001$) than during gold treatment (RR 1.7, $p = 0.365$). In this study we also looked for the degree of and the time of onset of proteinuria as variables. From these studies, it appears that the association between HLA DR3 and both nephrotic syndrome and early onset proteinuria was stronger compared with uncomplicated proteinuria and late onset proteinuria, respectively. Our data indicate that genetic factors in RA influence the development, the degree and the time of onset of drug induced proteinuria. In addition, they suggest that there may be differences in the pathogenesis of aurothioglucose- and d-penicillamine-induced proteinuria. Since this study indicated differences in HLA phenotype associations between AuTG- and DP-induced proteinuria, we investigated whether previous AuTG induced proteinuria is a risk factor for developing proteinuria during subsequent DP treatment, as stated by some authors.

In chapter 3, we report on patients with rheumatoid arthritis, who have been treated with AuTG and subsequently with DP, and who developed drug induced

proteinuria. Of these patients 12 developed Autg-induced and 19 DP-induced proteinuria. Of the 12 patients with AuTG-induced proteinuria, only 2 (17%) developed DP-induced proteinuria, indicating only a slightly increased risk as compared with the overall incidence (9.3%) of this reaction in DP-treated patients. In addition, only a minority (2 out of 19, 10.6%) of patients with DP-induced proteinuria had previous AuTG-induced proteinuria. HLA DR3 was present more frequently in DP-induced (50%) than in AuTG induced (21%) proteinuria. These data suggest that different mechanisms may be operative in AuTG- and DP-induced proteinuria. A practical consequence of this finding is, that in our opinion, a history of previous AuTG-induced proteinuria is insufficient reason to deny these patients the benefits of subsequent treatment with DP.

Most frightening of all side effects due to AuTG or DP are the hematotoxic reactions. We studied HLA phenotype frequencies in 21 rheumatoid arthritis patients with hematotoxic reactions, either thrombocytopenia or leucopenia, to AuTG or DP. Looking at the whole group of patients, antigens B8 and DR3 were significantly increased as compared to both RA patients without hematotoxicities and healthy controls. These contrasts were strongest in the patients with AuTG-induced thrombocytopenia or leucopenia: all patients developing either reaction to this drug, were B8- and/or DR3 positive ($p < 0.001$), 7 (78%) being positive for both antigens. In the patient group with DP induced reactions these antigens were also increased, but the differences were not significant. In the latter group, the prevalence of antigen DR4 was high, especially in the patient group with DP-induced thormbocytopenia, all 12 patients with this type of reaction being DR4 positive. From these studies we conclude that AuTG- and DP-induced hematotoxic side effects may have a different genetic background. These hematotoxic reactions seem to develop primarily (or even exclusively) in genetically predisposed RA patients. Comparing these associations with those in proteinuria on AuTGand DP, one can conclude that even for one type of drug, the genetic factor, in this case a particular HLA phenotype, need not to be the same for different types of side effects.

Another important question is whether not only drug toxicity but also type of clinical response is genetically co-determined.
In chapter 5 we report data on a cohort of 110 patients with RA, who were studied for a possible influence of HLA phenotypes on the reaction to parenteral gold in the first 6 months of treatment, in terms of both clinical re-

sponse and toxicity. The results indicated that frequencies of HLA B8 and DR3 were significantly increased in patients who responded excellently to gold treatment as compared with non-responders ($p = 0.04$ for both antigens). On the other hand HLA DR7 was increased in non-responders versus excellent and moderate responders ($p < 0.03$). Interestingly, drug toxicity was higher in excellent than in non-responders ($p = 0.04$), being exceptionally high in male excellent responders (85% versus 33% in females, $p = 0.019$),probably due to the increased frequency in B8 and DR3 in the ecellent responder group as a whole and the excellent responder males in particular.
It appears from these data that the HLA antigens B8 and DR3 who are associated with drug toxicity as shown above, also co-determine excellent clinical response to parenteral gold, whereas the presence of DR7 is associated with non-response. This is the first report of sex differences in reaction to parenteral gold, showing that toxicity is higher in male patients. This finding is probably related to the increased frequency of HLA B8 and DR3 in the male male patients of the group studied. The side effects found in this study were mainly skin rashes (early skin rashes) and showed a significant association with HLA B8.

The final part of this thesis reports studies on the reaction to either AuTG or DP in first degree relatives with RA treated with the same second line drug. Patients for these studies were obtained from a large family study project in our department. Chapter VI deals with clinical details of 2 pairs of sibs with definite rheumatoid arthritis, who responded remarkably similar to parenteral gold therapy, both in terms of toxicity and efficacy of the drug. Both pairs proved to be HLA identical. One of the pairs possessed the HLA antigen DR3, which has been associated with both drug toxicity and excellent clinical response (see above). The other pair did not possess this antigen, which suggests that the reaction to gold therapy in patients with rheumatoid arthritis may also be determined by other HLA or nearby located genetic factors. This observation initiated further studies. We managed to collect 13 pairs of first degree relatives with identically treated rheumatoid arthritis and studied concordances in terms of toxicity and clinical response to drug treatment. These selected 13 pairs had 15 paired therapeutic episodes on either AuTG or DP. Whereas statistically one should have expected 7.5 concordances for (non)toxicity, we found 10 concordant pairs. In addition, patients with a toxic relative developed more frequently drug toxicity than patients with a non-toxic relative (relative risk 1.8, chi square 2.117, $p = 1.46$).

On the other hand, favourable respondership in a relative did not seem to have any predictive value. We also looked for distribution of concordances over the three haplotype sharing categories (non, half and full HLA identical). Numbers, however, were too small to draw any conclusions. Studying the influence of sex on concordance showed that 4 out of 5 concordances for toxicity were in sex identical pairs, whereas 4 out of 5 concordances for non-toxicity were in sex non-identical pairs. Our data suggest that extending these investigations might reveal interesting information concerning family history as a risk factor.

Looking at HLA phenotypes as possible risk factors during treatment with aurothioglucose or d-penicillamine, the following can be said from our observations:

- antigens B8 and DR3: these antigens increased the risk for hematotoxic reactions on aurothioglucose and of proteinuria in d-penicillamine. They are also associated with excellent clinical response on aurothioglucose.
- antigen DR4: this antigen is a risk factor for the development of d-penicillamine-induced thrombocytopenia.
- Antigen DR7: this antigen is associated with non-response to aurothioglucose.

From the point of clinical use of aurothioglucose and d-penicillamine, we should like to state the following:

- the results of our studies do not recommend the use of HLA typing to influence the choice of second line antirheumatic drugs, as far as aurothioglucose and d-penicillamine are concerned.
- If the strong association between HLA phenotypes and hematotoxic reactions are confirmed by other studies, this would mean that tissue typing can identify among patients to be treated with aurothioglucose or d-penicillamine a small group at risk and a larger group with a negligible risk for hematotoxicity. This opens the posibility for individualized monitoring schedules, leading to more frequent monitoring of the patient at risk and less frequent monitoring of the group of patients with minimal risk to develop hematotoxicity. Since the latter group represent the larger part of patients to be treated, this could have a cost saving effect.

SAMENVATTING

De zogenaamde tweedelijns antireumatica, waaronder parenteraal goud en d-penicillamine, zijn van essentieel belang bij de behandeling van de ernstiger vormen van reumatoide artritis (RA). Belangrijke nadelen van deze therapie zijn het frequent voorkomen van bijwerkingen en de kans dat het middel onvoldoende effektief is. Dit proefschrift gaat over de mogelijke invloed van genetische faktoren op de wijze waarop patienten met RA reageren op deze behandeling. Bij dit onderzoek gebruikten wij HLA fenotypen, ook wel HLA antigenen genoemd, als genetische merkers. De resultaten van dit onderzoek wijzen erop, dat bepaalde bijwerkingen van bovengenoemde antireumatica geassocieerd zijn met bepaalde HLA fenotypen. Bovendien vonden wij dat het al of niet gunstig reageren op parenteraal goud, in deze studie aurothioglucose, eveneens mede bepaald wordt door bepaalde HLA fenotypen. Voor wat betreft de bijwerkingen: wij onderzochten met name die bijwerkingen, die gekwantificeerd kunnen worden, zoals proteinurie en hematologische bijwerkingen.

In hoofdstuk 2 worden de resultaten vermeld van een onderzoek naar de mogelijke associatie tussen HLA fenotypen en het ontwikkelen van proteinurie, ontstaan tijdens behandeling met aurothioglucose of d-penicillamine. Onze gegevens wijzen erop dat HLA DR3 meer voorkomt bij patienten met proteinurie, dan bij patienten die geen proteinurie ontwikkelden. Dit gold meer voor patienten, behandeld met d-penicillamine (RR 10.1, p = 0.001) dan voor patienten behandeld met aurothioglucose (RR 1.7, p = 0.365). In dit onderzoek bestudeerden wij ook de ernst van de proteinurie en het tijdstip van optreden als variabelen. Hieruit kwam naar voren, dat de associatie met HLA DR3 bij ernstige proteinurie (nefrotisch syndroom) en vroeg optredende proteinurie sterker was dan die met asymptomatische proteinurie en later optredende proteinurie. Onze gegevens wijzen er dus op, dat genetische faktoren het ontstaan, de ernst en het tijdstip van optreden van proteinurie beinvloeden. Aangezien ons onderzoek verschillen aangaf in HLA associatie tussen proteinurie t.g.v. aurothioglucose en d-penicillamine, hebben wij onderzocht of proteinurie t.g.v. aurothioglucose een risicofaktor is voor het ontwikkelen van deze bijwerking op d-penicillamine, zoals door sommige onderzoekers is gemeld.

Hoofdstuk 3 handelt over patienten met RA, die werden behandeld met aurothioglucose en in een latere fase met d-penicillamine, en die proteinurie op een

van deze of beide middelen ontwikkelden. Het betrof hier 12 patienten met proteinurie t.g.v. aurothioglucose en 19 met deze bijwerking t.g.v. d-penicillamine. Van de 12 patienten met proteinurie tijdens aurothioglucose, ontwikkelden er slechts 2 (17%) deze bijwerking tijdens d-penicillamine behandeling. Deze frequentie is niet veel groter dan die in een ongeselecteerde patientengroep behandeld met d-penicillamine (9,3%). Bovendien bleek slechts een klein gedeelte (2 van de 19, 10.6%) van de patienten met proteinurie op d-penicillamine vroeger dezelfde bijwerking op aurothioglucose te hebben gehad. Deze gegevens suggereren, dat de ontstaanswijze van proteinurie op aurothioglucose en d-penicillamine wel eens verschillend zou kunnen zijn. Een praktische consequentie van ons onderzoek is dat, naar onze mening, een proteinurie tijdens aurothioglucose geen reden mag zijn, de patient later een behandeling met d-penicillamine te onthouden.

Van alle bijwerkingen op aurothioglucose en d-penicillamine zijn de hematologische neveneffekten het meest gevreesd. Wij onderzochten HLA fenotype frequenties bij 21 patienten met reumatoide artritis, die hematologische bijwerkingen, hetzij trombocytopenie of leucopenie, op deze antireumatica hadden ontwikkeld (hoofdstuk 4). Voor de groep als geheel gold dat de HLA antigenen B8 en DR3 significant frequenter voorkwamen dan bij RA patienten zonder hematologische bijwerkingen. Dit verschil was het duidelijkst bij patienten met trombocytopenie of leucopenie t.g.v. aurothioglucose. Al deze patienten waren B8 en/of DR3 positief ($p < 0.001$). Bij de patienten met dezelfde soort bijwerkingen t.g.v. d-penicillamine kwamen deze HLA antigenen ook wel frequenter voor, maar de verschillen waren niet significant. Opvallend in deze groep was, dat het antigeen DR4 zeer vaak voorkwam; dit gold met name de groep patienten met trombocytopenie, die allen DR4 positief waren. De sterke HLA associaties bij hematologische bijwerkingen geven aan, dat genetische predispositie hier een belangrijke rol speelt. Net als bij de proteinurie (zie boven), valt op, dat de HLA associaties met hematologische bijwerkingen op aurothioglucose en d-penicillamine duidelijk verschillen.

Een andere belangrijke vraag is of ook het al of niet goed reageren op tweede lijns antireumatica beïnvloed wordt door genetische faktoren. Onderzoek, dat hierop betrekking heeft, wordt vermeld in hoofdstuk 5. Wij bestudeerden een cohort van 110 patienten met reumatoide artritis, gedurende de eerste 6 maanden van de behandeling met aurothioglucose. Uit dit onderzoek kwam naar voren, dat de frequenties van HLA B8 en DR3 significant groter waren bij pa-

tienten, die excellent reageerden op aurothioglucose, dan bij patienten die niet reageerden (non-responders) ($p = 0.04$ voor beide HLA antigenen). Daarentegen kwam HLA DR7 frequenter voor bij non-responders dan bij uitstekende - en matige responders ($p < 0.03$). Een interessante bevinding was, dat bijwerkingen frequenter voorkwamen bij uitstekende, dan bij non-responders ($p = 0.04$); dit was vooral het geval bij mannelijke patienten met een uitstekende respons (85% tegenover 33% bij vrouwelijke patienten, $p = 0.019$). Dit houdt mogelijk verband met de verhoogde frequentie van HLA B8 en DR3 in de uitstekende respondergroep als geheel en de mannelijke patienten uit deze groep in het bijzonder. Ons onderzoek wijst er dus op, dat de HLA antigenen B8 en DR3, die predisponeren tot het krijgen van bijwerkingen, ook mede bepalend zijn voor het uitstekend reageren op aurothioglucose, terwijl anderszijds de aanwezigheid van DR7 frequenter bij non-responders voorkomt. In ons onderzoek kon ook voor het eerst worden vastgesteld, dat er een geslachtsverschil bestaat in de reaktie op aurothioglucose, in die zin, dat bijwerkingen frequenter werden gezien bij mannelijke patienten. Deze bevinding houdt mogelijk verband met de toegenomen frequentie van HLA B8 en DR3 bij deze groep.

Het laatste deel van dit proefschrift betreft onderzoek naar de overeenkomsten en verschillen in reaktie op behandeling met hetzelfde tweedelijns antireumaticum, aurothioglucose of d-penicillamine bij eerste-graads verwanten met RA. Voor deze studies konden wij patienten selecteren uit een groot familiestudieprojekt. In hoofdstuk 6 beschrijven wij de klinische gegevens van 2 paren eerste-graads verwanten met RA, die opmerkelijk uniform reageerden op aurothioglucose, zowel wat betreft de bijwerkingen als de effektiviteit van het geneesmiddel. Het bleek dat beide paren HLA identiek waren. Bij één paar konden het HLA antigen DR3 worden aangetoond, een fenotype, dat predisponeert voor zowel bijwerkingen op als effektiviteit van aurothioglucose (zie boven). Het andere paar was negatief voor dit antigen, hetgeen suggereert, dat de reaktie op aurothioglucose bij patienten met RA ook nog door andere HLA of nabij gelegen genetische faktoren wordt bepaald. Deze waarneming vormde de aanleiding tot verdere studies. Wij waren in de gelegenheid 13 paren van eerste-graads verwanten op te sporen, die met hetzelfde tweedelijns antireumaticum behandeld waren. Bij deze paren bestudeerden wij de aan- of afwezigheid van concordanties, voor wat betreft het ontwikkelen van bijwerkingen en voor de effektiviteit van de behandeling. Bij deze 13 paren konden in het geheel 15 gepaarde therapeutische episoden worden bestudeerd, hetzij op aurothioglu-

cose of d-penicillamine. Op statistische gronden zou men bij deze 15 gepaarde waarnemingen 7.5 concordanties voor de aan- of afwezigheid van bijwerkingen hebben verwacht; het aantal gevonden concordanties was echter groter, namelijk 10. Verder bleek dat de frequentie van bijwerkingen groter was bij patienten met een familielid, die bijwerkingen had gehad op hetzelfde middel, dan bij patienten met een familielid, die geen bijwerkingen had gehad (RR 1.8, chi quadraat 2.117, p = 1.46). Een goede reaktie op goud of d-penicillamine bij een familielid bleek echter geen voorspellende waarde te hebben. Gezien het kleine aantal waarnemingen, kon geen uitspraak worden gedaan over de invloed van HLA haplotype sharing op de reaktie op de betreffende geneesmiddelen. Bij bestudering van de invloed van geslachtsidenticiteit op concordantie bleek, dat 4 van de 5 concordanties voor bijwerkingen werden aangetroffen bij geslachts identieke paren, terwijl 4 van de 5 concordanties voor de afwezigheid van bijwerkingen aanwezig waren in niet geslachtsidentieke paren. Onze waarnemingen suggereren, dat een gedetailleerde anamnese van de reaktie op tweedelijns antireumatica bij eerste-graads verwanten het perspectief biedt, in de toekomst de kennis betreffende familiair bepaalde faktoren op dit gebied uit te breiden.

Over HLA fenotypen als mogelijke risicofaktoren bij aurothioglucose - en d-penicillamine behandeling, kan het volgende gezegd worden:

- de antigenen B8 en DR3 verhogen het risico op hematologische bijwerkingen bij behandeling met aurothioglucose en op de proteinurie bij behandeling met d-penicillamine. Anderszijds zijn deze antigenen ook geassocieerd met een goede klinische respons op aurothioglucose.
- antigeen DR4 verhoogt het risico op trombocytopenie tijdens behandeling met d-penicillamine.
- antigeen DR7 vergroot de kans op een slechte respons op behandeling met aurothioglucose.

Uit het oogpunt van praktische toepasbaarheid zouden wij het volgende willen zeggen:

- op basis van onze resultaten kan HLA typering niet worden aanbevolen als mede bepalende faktor bij de keuze tussen aurothioglucose en d-penicillamine.
- de sterke associatie tussen bepaalde HLA fenotypen en het optreden van hematologische bijwerkingen, maakt verder onderzoek zinvol. Wanneer deze as-

sociaties inderdaad bevestigd kunnen worden, dan zou dit betekenen, dat middels HLA typering, een kleine risico dragende groep onderscheiden kan worden van een grote groep patienten met een vrijwel verwaarloosbaar risico voor het krijgen van hematologische bijwerkingen. Dit kan van praktisch nut zijn, omdat controleschema's dan meer geindividualiseerd zouden kunnen worden. Dit kan leiden tot meer adequate therapiecontrole en bovendien tot een aanzienlijke kostenbesparing.

ADDENDUM

MULTIPLE CASE FAMILY PEDIGREES

symbols used in the pedigrees

unaffected female/male

affected female/male

person with a history of polyarthralgias, normal physical and radiological findings

calendar age at the end of the follow up

interview and examination performed (end follow up)

IgM rheumatoid factor positive

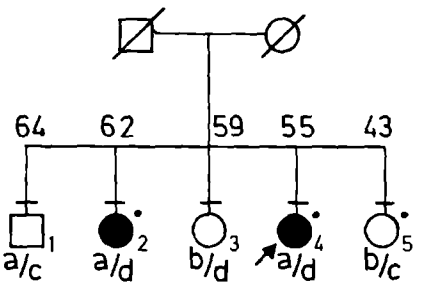
deceased

proband

family vd Wi

	son	mother
age of onset	21	58
disease course	seropositive polyarthritis during 14 years	seropositive polyarthritis during 8 years
treatment	parenteral gold (2 courses) d-penicillamine	parenteral gold
radiology	erosions of interphalangeal joints, mcp's and wrists; destruction of one hip	few erosions of mcp's and wrists
end follow up:		
articular findings	subluxation of mcp's, ulnar drift; deformities of feet; limited function of hands, wrists and one hip	no deformities normal functions
extra-articular findings	nodules	no
HLA phenotypes	A1Aw33B7B8DR5DRw8	A1A9B37B8DR5DRw10

Pedigree of family v d B

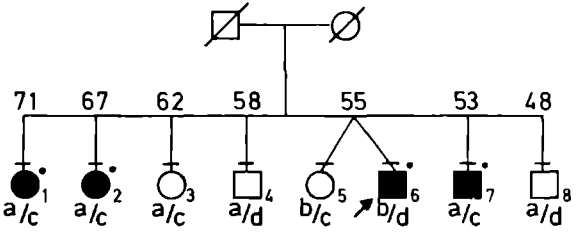


Haplotype a . A10 Bw 21 DR 3
,, b A2 B17 DR7
,, c A2 B15 DR4
,, d A3 B40 DR4

family vd B

	pedigree number	
	2	4
age of onset	53	52
disease course	active polyarthritis during 9 years	polyarthritis during 4 years
treatment	parenteral gold d-penicillamine, azathioprine and corticosteroids; knee arhroplasty	parenteral gold, hydroxychloroquine
radiology	erosions of interphalangeal joints, mcp's, mtp's, wrists, elbows	normal
end follow up:		
articular findings	active polyarthritis; limited motion of shoulders, elbows and hands	mild prolifrative synovitis of mcp's, limited motion of wrists and elbows
extra-articular finding	nodules	nodules

Pedigree of family H



Haplotype a A3 B37 DRw10
" b A1 B8 DR1
" c A2 B15 DR4
" d A11 B27 DR1

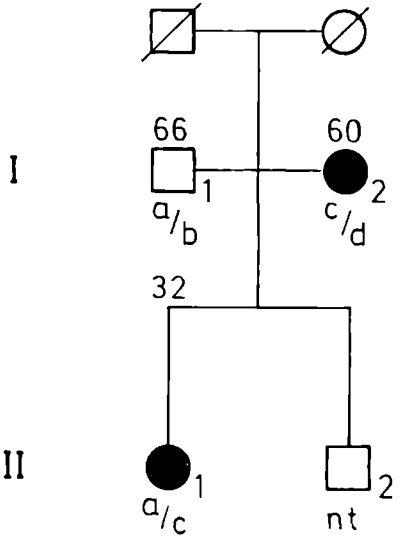
family H

	pedigree number			
	1	2	6	7
age of onset	50	60	48	51
disease course	active disease during 20 years	mild polyarthritis during 7 years	mild polyarthritis during 7 years, pleurisy	arthralgias (symmetrical) during 2 years
treatment	hydroxychloroquine parenteral gold corticosteroids 2 knee arthro-plasties	parenteral gold	hydroxychloroquine parenteral gold d-penicillamine	no medication
radiology	extensive erosions in hands, wrists, elbows, shoulders and feet; cervical subluxation	normal	few erosions of mcp's	normal
end follow up:				
articular findings	severe deformities and impaired functions of all peripheral joints	mild synovitis of wrists, normal functions, no deformities	no active joints, normal functions	normal
extra-articular findings	nodules	no	extensive nodules, pleurisy	no

family Key

	sister	brother
age of onset	72	53
disease course	seropositive polyarthritis during 6 years	seronegative polyarthritis during 7 years
treatment	hydroxychloroquine parenteral gold (2 courses)	hydroxychloroquine parenteral gold (2 courses)
radiology	erosions of wrists and mtp's; coxarthrosis and gonarthrosis	erosions of wrists, pip's elbow
end follow up:		
articular findings	no disease activity	moderate disease activity arthritis of wrists and mcp's
extra-articular findings	no	no
HLA phenotypes	A2B7DR4/A2B18DR4	A2B7DR4/A2B18DR4

Pedigree of family van der E.

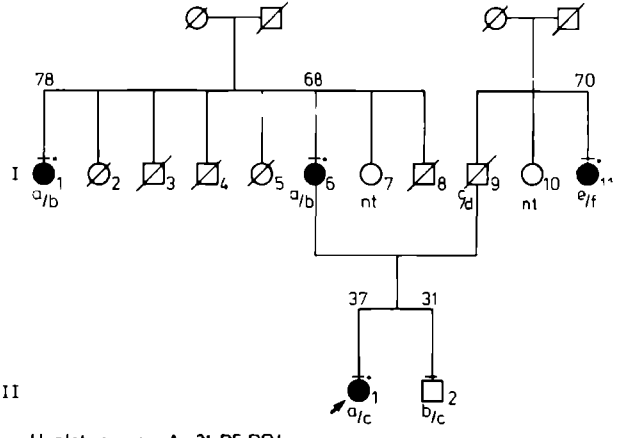


Haplotype a: A1 B8 DR3
,, b A28 Bw22 DR4
,, c: A2 B15 DRw6
,, d A1 B27 DR1

family vd E

	pedigree number	
	I.2	II.1
age of onset	54	28
disease course	polyarthritis during 21 years with cervical involvement; Hodgkin's disease	polyarthritis during 5 years of wrists, mtp's and hips
treatment	parenteral gold	parenteral gold, d-penicillamine arthroplasty of one hip
radiology	erosions of mcp's and wrists; severe subluxation of atlanto-axial joint with fracture of the dens	coxitis; bilateral sacroiliitis; erosions of atlanto-axial joint
end follow up:		
articular findings	limited motions of wrists proliferative synovial swelling of mcp's	mild synovitis of peripheral joints, limited painful motion of hip, back and cervical spine
extra articular findings	no	no

Pedigree of family Lo

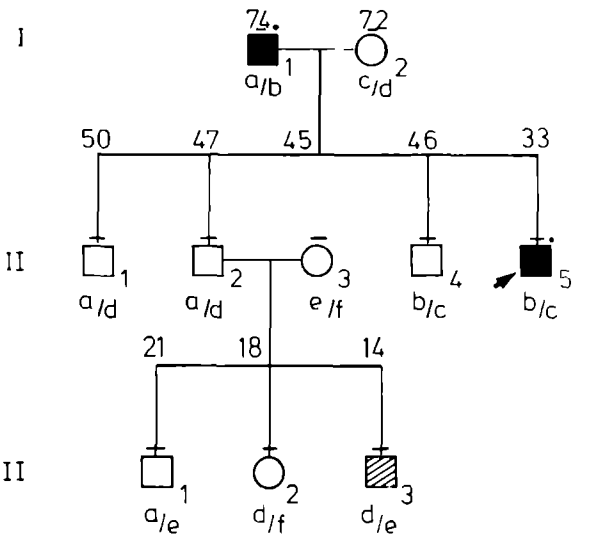


Haplotype	a	Aw31	B5 DR4
..	b	A3	B18 DR3
..	c	A9	B37 DR4
..	d		?
Phenotype e/f		A2 A11 B7 Bw22 DR1 DR2	
I 5 and II 2		type 1 diabetes mellitus	

family Lo

	pedigree number			
	I.1	I.6	I.11	II.1
age of onset	56	44	45	23
disease course	longstanding active polyarthritis	longstanding active disease	25 years of active polyarthritis	13 years of active polyarthritis
treatment	hydroxychloroquine, corticosteroids, cyclophosphamide	parenteral gold, (2 courses), corticosteroids, synovectomy	hydroxychloroquine	hydroxychloroquine, parenteral gold (2 courses), d-penicillamine azathioprine
radiology	erosions in interphalangeal joints, mcp's and mtp's	erosions in pip's mcp's, mtp's and wrists	erosions in mcp's and wrists	extensive erosions in mcp's, wrists, mtp's, shoulders and knees
follow up:				
articular findings	no disease activity, deformities of hands and feet; impaired functions of hands	no disease activity, deformities of hands and feet; impaired functions	no disease activity, ulnar drift; limited motions of wrists	active synovitis of mcp's, wrists and knees
extra-articular findings	no	no	no	no

pedigree of family Waa



Haplotype	a	A 3 B 7 DRw 6
,,	b	A 10 B 27 DR 1
,,	c	A 2 B 15 DR 4
,,	d	Aw 32 B 27 DR 1
,,	e	A 28 Bw 35 DRw 8
,,	f	A 2 B 12 DR 4

family Waa

	pedigree number			
	I.1	II.5	III.2	III.3
age of onset	52	26	20	8
disease course	longstanding polyarthritis	six years of active polyarhritis	stiffness and pain of the axial skeleton	six months of recurrent fever with polyarthritis
treatment	parenteral gold (2 courses), corticosteroids; knee arthroplasty	parenteral gold (2 (2 courses),corticosteroids, d-penicillamine, azathioprine	no	salicylates
radiology	erosions in mcp's, wrists, knee and mtp's	extensive erosions pip's, mcp's, mtp's schoulder, knees; atlanto-axial instability	bilateral sacroiliitis	not done
end follow up:				
articular findings	no disease activity, deformities of hands, impaired function	active disease; impaired function of hands and knees	signs of axial arthritis	normal
extra-articular findings	no	nodules	no	no

Pedigree of family Thie

Haplotype a A3 B7 DRw10
b A9 Bx DR2
c A2 B15 DR4
d A2 B12 DR4

family Thie

	pedigree number						
	2	4	5	9	10	11	15
age of onset	50	54	no data available	45	59	53	36
disease course	polyarthritis during 5 years, followed by complete remission	polyarthritis during 11 years		polyarthritis during 5 years followed by a remission of 10 years years	recent onset of poly-arthritis	recent onset of poly-arthralgias	polyarthritis during 6 years followed by complete remission
treatment	hydroxychlo-roquine	hydroxychloro-quine, parenteral gold (2 courses) corticosteroids		hydroxychloro-quine, parenteral gold (2 courses)	analgesics	analgesics	parenteral gold
radiology	not done	erosions in mcp's, mtp's and wrists		erosions in mcp's mtp's and wrists	normal	normal	normal
end follow up:							
articular findings	normal	active polyarthr-itis; deformities and impaired func-tion of hands		normal	mild poly-arthritis, normal func-tions	joint tender-ness, no evident swel-ling, normal functions	normal
extra-articular findings	no	nodules		no	no	no	

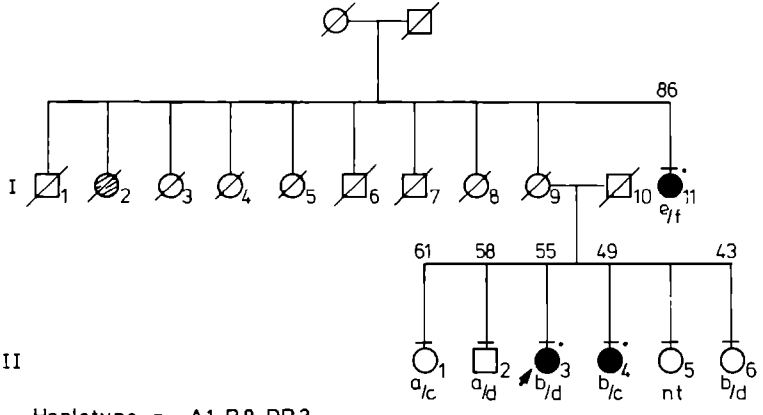
Pedigree of family VIa

Haplotype	a	A2	Bw22 DR4
,,	b	A1	B8 DR3
,,	c	A2	B12DR5
,,	d	A1	B8 DR3

family VIa

	pedigree number	
	1	4
age of onset	46	29
disease course	active polyarthritis during 21 years	polyarthritis during 4 years followed by complete remission
treatment	parenteral gold, corticosteroids d-penicillamine, azathioprine, cyclophosphamide; knee arthroplasty	hydroxychloroquine, parenteral gold
radiology	extensive erosions of peripheral joints including shoulders and hips; atlanto-axial subluxation	normal
end follow up:		
articular findings	active polyarthritis with marked residual damage	normal
extra-articular findings	nodules, vasculitis, Felty's syndrome	no

Pedigree of family Smi

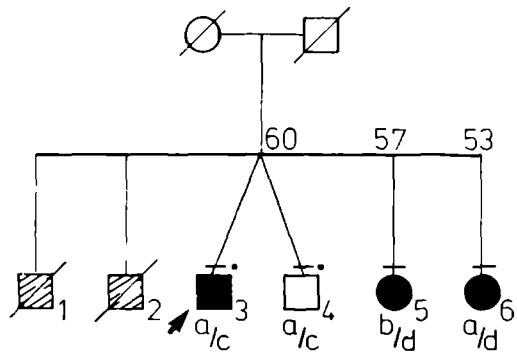


Haplotype	a	A1 B8 DR3
,,	b	A3 B15 DR1
,,	c	A3 B7 DR2
,,	d	A28 B40 DR4
Phenotype e/f		A1 A3 B27 B8 DR1 DR3

family Sm1

	pedigree number		
	I.11	II.3	II.4
age of onset	45	28	45
disease course	longstanding polyarthritis of hands, feet, knees	longstanding polyarthritis of peripheral joints	polyarthritis during 5 years
treatment	no treatment	hydroxychloroquine, parenteral gold, d-penicillamine	parenteral gold, d-penicillamine
radiology	not done	extensive erosions of interphalangeal joints, mcp's, mtps's and wrists	few erosions of feet. mild instability of atlanto-axial joint
end follow-up:			
articular findings	active synovitis of knees; deformities of hands and feet	no disease activity; deformities of hands, impaired function	no disease activity normal function of hands, subluxation of mtp's
extra-articular findings	no	nodules	no

Pedigree of family Ko

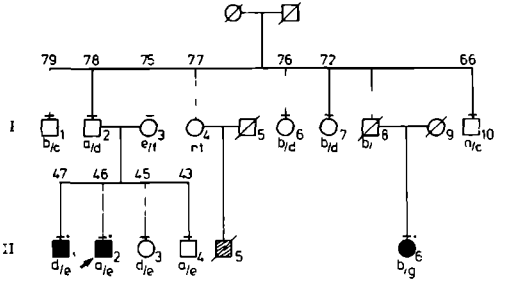


Haplotype	a	A 3	B37	DRw10
,,	b	A11	Bw22	DRw6
,,	c	A1	B8	DR3
,,	d	A2	B12	DR4

family Ko

	pedigree number		
	3	5	6
age of onset	47	53	49
disease course	progressive disease during 12 years, leading to destruction of all peripheral joints; severe cervical involvement	polyarthritis during 3 years	polyarthritis during 3 years
treatment	parenteral gold (2 courses) d-penicillamine, cortico-steroids; arthroplasties knee, hip, 2 elbows; cervical spon-, dylodesis	parenteral gold	hydroxychloroquine parenteral gold d-penicillamine
radiology	destruction of all peripheral joints; atlanto-axial involvement	one erosion	few erosions in mtp's
end follow up:			
articular findings	severe disability; still active disease	no disease activity; normal functions	no disease activity normal functions
extra-articular findings	nodules, pleurisy	no	no

Pedigree of family Ja

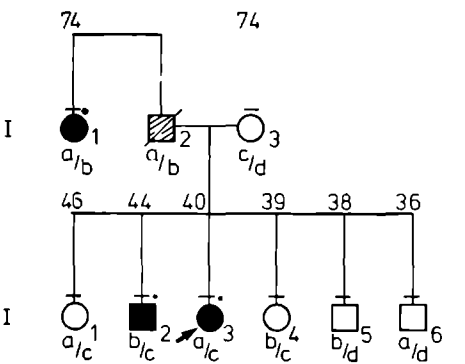


Haplotype	a	A1 B8 DR4
..	b	A2 B15 DR6
..	c	A2 B15 DR4
..	d	A9 B15 DR4
..	e	A1 B8 DR3
..	f	A1 B8 DR7
..	g	A2 B17 DR7

family Ja

	pedigree number		
	II.1	II.2	II.6
age of onset	33	31	28
disease course	polyarthritis during 12 years	mild polyarthritis during 8 years followed by remission, Caplan's syndrome	chronically active polyarthritis, psoriasis
treatment	parenteral gold d-penicillamine	d-penicillamine	d-penicillamine
radiology	erosions of pip's, mcp's mtp's, wrists, shoulders	few erosions in wrists	erosions in mcp's, mtp's and wrists
end follow up:			
articular findings	active polyarthritis, ulnar drift	joint tenderness, no swellings, no deformities, normal functions	active synovitis mcp's, one elbow, one knee
extra-articular findings	nodules	no	no

Pedigree of family van deV



Haplotype	a	A2	B7 DR 2
..	b	A11	B7 DR 3
..	c	A2	B7 DRw6
..	d	A3	Bw35 DR1

family van de V

	pedigree number		
	I.1	II.2	II.3
age of onset	65	38	33
disease course	polyarthritis during 9 years	active polyarthritis during 6 years	mild polyarthritis followed by complete remission
treatment	analgesics	parenteral gold, d-penicillamine, corticosteroids	d-penicillamine
radiology	few erosions in hand and feet	extensive erosions of mcps's, mtp's and wrists	few erosions in wrists
end follow up:			
articular findings	mild synovitis of 2 ip's, normal function	active polyarthritis, deformities of hands, limited motion of wrists	normal
extra articular findings	no	nodules	no

De schrijver van dit proefschrift werd op 21 juli 1940 geboren te Haskerdijken.
Na een opleiding tot onderwijzer en vervulling van de militaire dienstplicht, werd in 1965 het diploma HBS-B gehaald aan de Rijks HBS te Sneek.
Studie Geneeskunde aan de Gemeentelijke Universiteit te Amsterdam, in 1974 afgesloten met het artsexamen. Opleiding Interne Geneeskunde in achtereenvolgens Enschede, Ziekenhuis De Stadsmaten en vanaf 1977 het St. Radboudziekenhuis te Nijmegen. Vanaf 1980 werkzaam als wetenschappelijk medewerker op de afdeling Reumatische Ziekten van het St. Radboudziekenhuis te Nijmegen. Sinds 1 juli 1984 als reumatoloog verbonden aan het Wilhelmina Ziekenhuis te Assen en het Refaja Ziekenhuis te Stadskanaal.

STELLINGEN

I

De reactie op langwerkende antireumatica bij patienten met reumatoïde arthritis wordt medebepaald door HLA antigenen.

II

De sterke associaties tussen bepaalde HLA antigenen en haematologische bijwerkingen op goud en d-penicillamine wekken de verwachting dat weefseltypering in de toekomst een kostenbesparende factor kan worden in de behandeling van reumatoïde arthritis.

III

De HLA antigenen B8 - DR3 verhogen niet alleen het risico op bijwerkingen maar beïnvloeden tevens het tijdstip waarop en de mate waarin de reactie optreedt.

IV

De observatie dat de HLA antigenen B8 - DR3 wèl een rol spelen bij het ontstaan van proteïnurie op d-penicillamine en niet bij proteïnurie op goud kan betekenen dat de pathogenese van deze reactie op beide middelen verschilt.

V

Bij vergelijking van HLA associaties tussen identieke reacties op goud en d-penicillamine moet rekening gehouden worden met het relatief hoge aantal vroege uitvallers als gevolg van dermatitis tijdens goudbehandeling.

VI

De hogere frequentie van de HLA antigenen B8 - DR3 in de mannelijke patientengroep met reumatoïde arthritis maakt het zinvol om in de toekomst bij HLA studies geslachtsspecificatie te betrekken.

VII

Het feit dat bepaalde extra-articulaire manifestaties bij reumatoïde arthritis sterk geassocieerd zijn met de HLA antigenen B8 - DR3 en DR4 kan er op wijzen dat het risico op een slechtere lange termijnprognose bij patienten met deze phenotypen verhoogd is.

Dinant et al: Arthritis Rheum 1980, 1336
Cunningham et al: Reumatol Int 1982,2: 137-139

VIII

HLA haplotype sharingstudies bij eerstegraads verwanten met reumatoïde arthritis hebben tot nu toe geen verheldering gebracht omtrent de invloed van genetische factoren op het ontstaan van deze ziekte.

o.a. Kahn et al: Tissue Antigens 1983:22, 182-185

IX

Het lijkt zinvol om de familiegegevens van patienten met reumatoïde arthritis bij wie behandeling met goud of d-penicillamine wordt overwogen, uit te breiden met informatie over de reactie op deze middelen bij eerstegraads verwanten.

X

De overeenkomsten in klinisch-immunologische manifestaties tussen lepra en reumatoïde arthritis zouden verder in betekenis winnen indien deze terug te voeren zouden zijn tot een gemeenschappelijke genetische basis.

Panayi : Ann Rheum Dis 1982: 41, 102-103
van Eden et al : Human Immunol 1982: 4, 343-350

XI

Weefseltypering als diagnostisch hulpmiddel bij reumatoïde arthritis dient ontraden te worden.

XII

In de totale kosten van de behandeling met intramusculair goud is de goudprijs een te verwaarlozen factor.

Liang et al: J Rheumatol 1976: 5,241-243

XIII

Mannelijke patienten met reumatoïde arthritis roken méér en hebben daardoor waarschijnlijk een verhoogde kans op het krijgen van longkanker.

eigen waarneming

XIV

De aanvankelijke vrees dat de introductie van azathioprine bij de behandeling van reumatoïde arthritis zou leiden tot een verhoogd risico op lymforeticulaire maligniteiten is tot op heden door retrospectief onderzoek niet bewaarheid.

o.a. eigen waarneming

XV

Het"prednisolonkuurtje" zoals bij exacerbatie van asthmatische bronchitis niet ongebruikelijk is, moet bij opvlamming van chronische arthritis ontraden worden.

XVI

Bij de diagnostiek van gewrichtsziekten zijn klinische symptomen belangrijker dan de aan- of afwezigheid van serologische afwijkingen.

XVII

In de "top tien" van serologische bepalingen bij reumatische ziekten dient de AST van de eerste- naar de laatste plaats te zakken of te verdwijnen.

Nijmegen, 18 december 1985 F.Speerstra