

HLA-DRB1 typing in rheumatoid arthritis: predicting response to specific treatments

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Abstract

Objective—To determine the predictive value of shared epitope alleles for response to treatment in patients with rheumatoid arthritis.

Methods—Patients from our previously published triple DMARD study were tested for the presence of shared epitope alleles (DRB1 *0401,0404/0408, 0405,0101, 1001, and 1402). Patients who were shared epitope positive were then compared with those who were negative to see if there was a differential effect on therapeutic response.

Results—Shared epitope positive patients were much more likely to achieve a 50% response if treated with methotrexate-sulphasalazine-hydroxychloroquine compared with methotrexate alone (94% responders versus 32%, $p < 0.0001$). In contrast shared epitope negative patients did equally well regardless of treatment (88% responders for methotrexate-sulphasalazine-hydroxychloroquine versus 83% for methotrexate). Additionally, a trend toward an inverse relation of the gene dose was seen for response to methotrexate treatment ($p = 0.05$).

Conclusions—These data suggest that determining shared epitope status may provide clinical information useful in selecting among treatment options.

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severe clinical manifestations. We have recently reported on the superior efficacy of the combination of hydroxychloroquine, sulphasalazine, and methotrexate, as compared with methotrexate alone or hydroxychloroquine plus sulphasalazine in a double blind, randomised, controlled study, documenting clinical response over a two year treatment span.⁸ In that study, 77% of the patients treated with combination therapy had a marked clinical improvement, compared with 33% of the patients treated with methotrexate alone. The availability of successful combination treatments provides an impetus to start treatment earlier in the course of disease before severe manifestations occur. However, no standard assessment approach distinguishes patients likely to respond to monotherapy (for example, methotrexate) from those who are more suited to combination treatments.

Although the aetiology of RA is unknown, there is a strong genetic association between particular HLA-DR alleles and severe, progressive forms of the disease. In numerous studies of various populations, HLA-DRB1 alleles that contain a similar sequence at amino acid positions 67–74 in the third hypervariable region of the DR molecule (called the “shared epitope”) are associated with RA, including DRB1*0401, *0404/*0408, *0405, *0101, *1001, and *1402 (reviewed in reference 9). In North American white people, the DRB1*0401 and *0404 alleles are the most prevalent, and these genes are associated with the highest risk of severe disease.^{9–12}

People with these alleles have an incidence of RA five times to seven times higher than people without these alleles; however, as these alleles are frequent in white populations, most people with these genes do not have RA, and the use of HLA typing for screening the general population for disease risk is therefore not practical.¹³ On the other hand, the presence of these alleles predisposing to severe, progressive disease potentially serves as a marker for poor prognosis of patients known to have RA. Within the spectrum of clinical manifestations, prediction of the disease's natural history and of the expected response to specific treatments may be improved by patient stratification for genetic and clinical markers indicating likelihood of severe, progressive disease.

Using patients from the clinical study mentioned above, we have now tackled the question of whether the response to treatment in patients with RA differs based on the presence of HLA-DRB1 alleles.

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Rheumatoid arthritis (RA) is a chronic disease, with a spectrum of clinical manifestations characterised by synovial inflammation that leads to progressive joint damage, disability, and increased mortality.^{1–2} Conventional treatment for RA is essentially an empiric choice of disease modifying antirheumatic drugs (DMARDs) such as gold, hydroxychloroquine, sulphasalazine, d-penicillamine, azathioprine, and methotrexate; all these drugs have been shown to be effective individually, at least in the short-term.^{3–5} Regrettably, with the exception of methotrexate, the long term efficacy of these drugs is less than ideal, so most patients are no longer taking them after two years because of toxicity or lack of efficacy.^{6–7} There is a need to identify additional therapeutic options that are both effective and well tolerated by individual patients. Various combination treatments in which multiple drugs are used simultaneously are under evaluation for improved clinical efficacy in an attempt to modulate the course of the disease more effectively in patients with

Table 1 Baseline characteristics of patients, according to study group

Characteristic	Methotrexate (n=28)	Sulphasalazine and hydroxychloroquine (n=30)	All three drugs (n=26)
Age (y)			
mean	50	49.8	50.4
range	26-69	3-63	27-67
Sex (F/M)	18/10	21/9	16/10
Rheumatoid factor (% positive)	89	86	88
DMARDs previously used	1.5	1.5	1.4
Current prednisone therapy (%)	54	47	50
Shared epitope (% positive)	79	73	69

Methods

GENERAL

The Rheumatoid Arthritis Investigational Network (RAIN) conducted this study in collaboration with the Virginia Mason Research Center. RAIN brings the rheumatologists at the University of Nebraska together with rheumatologists in Nebraska, Iowa, South Dakota, Minnesota, and Illinois who are interested in clinical studies in RA. All physicians who participate in this network not only are involved with patient enrollment and data collection, but also have aided in the development of the study protocols themselves.

PATIENT SELECTION

We asked patients who met criteria for this study to participate; they included those followed up in the rheumatology clinic at the University of Nebraska Medical Center, the Omaha Veterans Administration Medical Center, or in the private offices of network physicians. All patients enrolled signed an informed consent document approved by the Institutional Review Board.

Eligibility criteria for the original study as previously reported⁸ included active RA, previous treatment failure with at least one DMARD, and no previous treatment with combinations of the study drugs.

EXPERIMENTAL DESIGN

We enrolled 102 patients in the original two year, double blind, randomised, controlled study. The experimental design of the original study has been reported elsewhere.⁸ Briefly patients were randomised to one of three treatment groups: Group 1, methotrexate only; Group 2, hydroxychloroquine and sulphasalazine; Group 3, all three active compounds (table 1).

Patients were followed up by blinded evaluators until they completed the protocol (two years), until side effects occurred, or until they failed because of lack of efficacy. The major end point of our study was 50% improvement based on fulfilling three of the following requirements (modified Paulus¹⁴): (1) morning stiffness < 30 minutes or improved by 50%; (2) joint tenderness improved by 50%; (3) joint swelling improved by 50%; (4) sedimentation rates < 30 mm 1st h for women or < 20 mm 1st h for men. If patients did not meet these 50% improvement criteria at all of the three month evaluation periods after they had received maximal therapy (nine months), they were considered an efficacy failure. Using this end point, patients were classified as successful

completers (50 patients) or efficacy failures (37 patients). An additional 13 patients were withdrawn because of medication side effects, and two patients did not complete because of protocol violations.

Blood was drawn for DNA analysis from 94 of 102 patients and included 86 of 87 patients (99%) that could be analysed for efficacy (successful completers or efficacy failures). Because of the differences in frequency of the shared epitope among races, only white patients (n=98) who were completers or who were efficacy failures were included in the subsequent analysis (n=84). All DNA analysis was done by investigators blinded to the results of both the group assignment and the results of treatment.

HLA-DRB1 ANALYSIS

DNA was extracted from ficoll separated peripheral blood lymphocytes and amplified by the polymerase chain reaction. For determination of DR4 alleles, group specific biotinylated primers for DRA and DRB1*04 were used simultaneously. DR4 subtype was detected by subsequent hybridisation with allele specific oligonucleotide probes specific for the shared epitope region.¹⁵ Detection of the coamplified DRA gene served as an internal standard and was included as a technical control in each sample. Probes used for hybridisation were immobilised on a solid support, and presence of the amplified product was measured using streptavidin conjugated horseradish peroxidase, using a semi-automated GeneMAT_{RA} DNA analysis system (Cypress Technologies). Other HLA alleles were determined by standard DNA based, non-radioactive methods.¹⁶

STATISTICAL ANALYSIS

The primary end point of the original study was successful completion of the two year protocol. Differences among the three treatment groups in completers and efficacy failures were analysed using both Fisher's exact test and the log rank test.¹⁷ In this study Fisher's exact test was used to compare the effects of shared epitope status in the three different treatment groups.

The differential effect of treatment group on the magnitude of the change in tender joint and swollen joint scores based on shared epitope status was also determined by the Fisher's exact test. The effect of gene dose on response to treatment was analysed by the analysis of variance.

Results

Eighty four white RA patients finished the two year study as either "successful completers" or "efficacy failures." Samples from these patients were tested for the presence of the RA associated alleles (shared epitope). Of these patients, 74% were positive for at least one shared epitope allele; while 29% were positive for some combination of two shared epitope alleles. These included 51% of patients positive for DRB1*0401 (Dw4), 19% positive for *0404/*0408 (Dw14), 19% positive for *0101(DR1), 5% positive for *1001(DR10), and 5% positive for *0405 (Dw15). No *1402

Table 2 Effect of shared epitope on treatment response

Shared epitope status	Treatment group						p Value†		
	Methotrexate		Sulphasalazine-hydroxychloroquine		All three drugs		MTX v triple	S and H v triple	MTX v S and H
	Successful completers	Efficacy failures	Successful completers	Efficacy failures	Successful completers	Efficacy failures			
Positive	7 (32%)	15	10 (45%)	12	17 (94%)	1	<0.001	<0.002	0.35
Negative	5 (83%)	1	3 (38%)	5	7 (88%)	1	0.69	0.06	0.09
p Value*	<0.04		0.52		0.53				

*p Value for comparisons of completers based on epitope status within each treatment group.

†p Value for comparison of treatment groups with the same epitope status.

(DR14) positive patients were found in this white population. The predictive value of these genes for response to treatment was assessed for patients receiving methotrexate alone, sulphasalazine-hydroxychloroquine, or all three drugs together.

As table 2 shows, the frequency of successful completion of the clinical trial showed marked differences among patients stratified by shared epitope status. Among patients receiving methotrexate alone, only 32% of the shared epitope positive patients successfully completed the study; on the other hand, 94% of shared epitope positive patients in the three drug group were successful completers ($p < 0.001$). The response of shared epitope positive patients treated with all three drugs was also significantly better than those treated with sulphasalazine-hydroxychloroquine (45% response, $p < 0.002$).

Within the methotrexate treatment group, fewer shared epitope positive patients were successful completers compared with patients without the shared epitope ($p < 0.04$). This was not true for patients in the other treatment groups: fewer than half of the patients treated with sulphasalazine-hydroxychloroquine were successful completers, regardless of shared epitope status; whereas, most of the patients treated with all three drugs did well, regardless of shared epitope status.

Among shared epitope negative patients, those treated with all three drugs showed no difference in response rate from those treated with methotrexate alone (88% versus 83% respectively, $p = \text{NS}$) but did respond better than those treated with sulphasalazine-

hydroxychloroquine (88% versus 38% respectively, $p < 0.02$).

To assure that these findings were not based in some way on the fact that we had in our original study chosen to define success as 50% improvement (rather than 20%, 60%, or any other degree of improvement), we calculated the change in the tender and swollen joint scores (nine month scores minus initial scores) for patients based on treatment group and epitope status. As table 3 shows, shared epitope negative patients fared equally well regardless of whether they were treated with methotrexate or triple therapy. Shared epitope positive patients, however, improve much more if treated with triple therapy ($p = 0.007$ and 0.019).

The poor therapeutic response of the methotrexate treated shared epitope positive patients was further corroborated by analysis of the effect on dose of the shared epitope. As table 4 shows, there was a trend toward an inverse relation of gene dose on response to methotrexate ($p = 0.05$). This was not seen in the sulphasalazine-hydroxychloroquine group or in the group that received all three drugs.

The presence of rheumatoid factor, the duration of disease, and the number of DMARDs previously used were not predictive of response to treatment in this study.

Discussion

The choice of which of several available DMARDs to use in treating RA patients is an important dilemma faced by the rheumatologist. Currently, this is an empirical decision, and no laboratory or clinical assessments have predicted an individual patient's response to a particular drug or combination of drugs. There is a growing consensus that the earlier the appropriate treatment can be started, the better the outcome.^{3, 18} DMARDs are generally slow acting, and joint damage progresses while the appropriate drug is sought.

Currently, methotrexate is the initial DMARD of choice for most rheumatologists.¹⁹ However, remissions are rare^{21, 22} and only about 35% of methotrexate treated patients will meet 50% improvement criteria.^{8, 22, 23} We have recently demonstrated that the combination of methotrexate, sulphasalazine, and hydroxychloroquine is significantly more effective than methotrexate alone in treating patients with RA (77% versus 33% efficacy at two years using 50% improvement criteria).⁸ However, as some patients do well with methotrexate alone, the role of triple therapy in individual

Table 3 Degree of clinical improvement stratified by shared epitope status

Treatment group	Shared epitope status	Change in tender joints	Change in swollen joints
Methotrexate	Negative	-19.5 (1.4)	-19.2 (10.1)
All three drugs	Negative	-20.0 (21.0)	-19.4 (15.1)
Methotrexate	Positive	-12.5 (14.7)	-15.0 (15.4)
All three drugs	Positive	-23.4 (9.1)	-25.9 (11.1)

Values are mean (SD) of the change in scores from start of treatment until study completion.

Table 4 Shared epitope dose effect: patients with 50% response

	Double dose	Single dose	None	p Value
Methotrexate (%)	2/8 (25)	5/14 (36)	5/6 (83)	0.05
Sulphasalazine-hydroxychloroquine (%)	4/8 (50)	6/14 (43)	3/8 (38)	1.0
All three drugs (%)	5/5 (100)	12/13 (92)	7/8 (88)	1.0

*p Values are for dose effect of each treatment.

patients is unclear. A reliable predictor of response to specific treatments would greatly aid in the early application of appropriate therapy for RA.

Many studies have confirmed the strong association of particular HLA alleles with RA.²⁴⁻²⁷ The shared epitope, a sequence stretch in the third hypervariable region of the DRB1 molecule, has been most closely associated with this disease.^{28,29} This sequence is found on several DR4 positive alleles (*0401, *0404, *0405, etc), as well as on some non-DR4 alleles (*0101, *1001, etc). Studies in white populations have generally agreed that the DR4 shared epitope positive alleles confer susceptibility for a more severe disease course, while alleles negative for the DR4 shared epitope are associated with milder disease.^{10,11,30-32} We chose to analyse shared epitope positive allele status among the patients who participated in a recent therapy trial, to determine whether this might predict response to specific treatment. Patients were typed using a DNA based, sequence specific, oligonucleotide probe assay for the alleles DRB1*0401, *0404/*0408, *0405, *0101, *1001, and *1402 without knowledge of their treatment group or outcome.

A striking differential response to treatment in RA patients was observed, based on their shared epitope status. While most patients in the triple therapy arm of the study did well regardless of HLA type, those treated with methotrexate alone showed a marked difference; 83% of those patients who were shared epitope negative were successful completers, while only 32% of those who were shared epitope positive responded successfully. Looked at a different way, among patients positive for the shared epitope, 94% were successful completers if treated with triple therapy, but 68% were efficacy failures if treated with methotrexate alone. These results are consistent with reports that shared epitope positive patients are those at greatest risk for severe, erosive disease; it seems probable that such patients require more aggressive treatment to achieve a sustained clinical response. Similarly, patients negative for the shared epitope have been reported to have milder disease; these patients generally did well during the two year study period with methotrexate alone (83% successful completers). Our results were similar if only DR4 associated share epitope positive patients were considered, which leaves open the question of whether non-DR4 associated alleles are important for prediction of response to treatment.

Joint inflammation multiplied by time equals increased joint damage in RA; thus, "time is cartilage," and earlier use of effective treatments is essential for optimum joint function and patient management. While many options are available when selecting DMARD therapy for patients with RA, it often takes one to six months before efficacy is seen, highlighting the importance of making therapeutic choices as soon as possible. As responses to any single DMARD differ among individual patient, many patients follow a disease course that may

progress without sustained therapeutic response over several years, with resultant joint destruction. While predictive tools are necessarily imprecise, we are encouraged that the HLA markers studied in this report appear to be specific markers of response to treatment in a rigorous long term clinical trial protocol. Such genetic analysis may provide an assessment tool to aid in choosing early treatment for patients with RA, thereby helping to prevent or delay some of the morbidity and mortality associated with this disease.

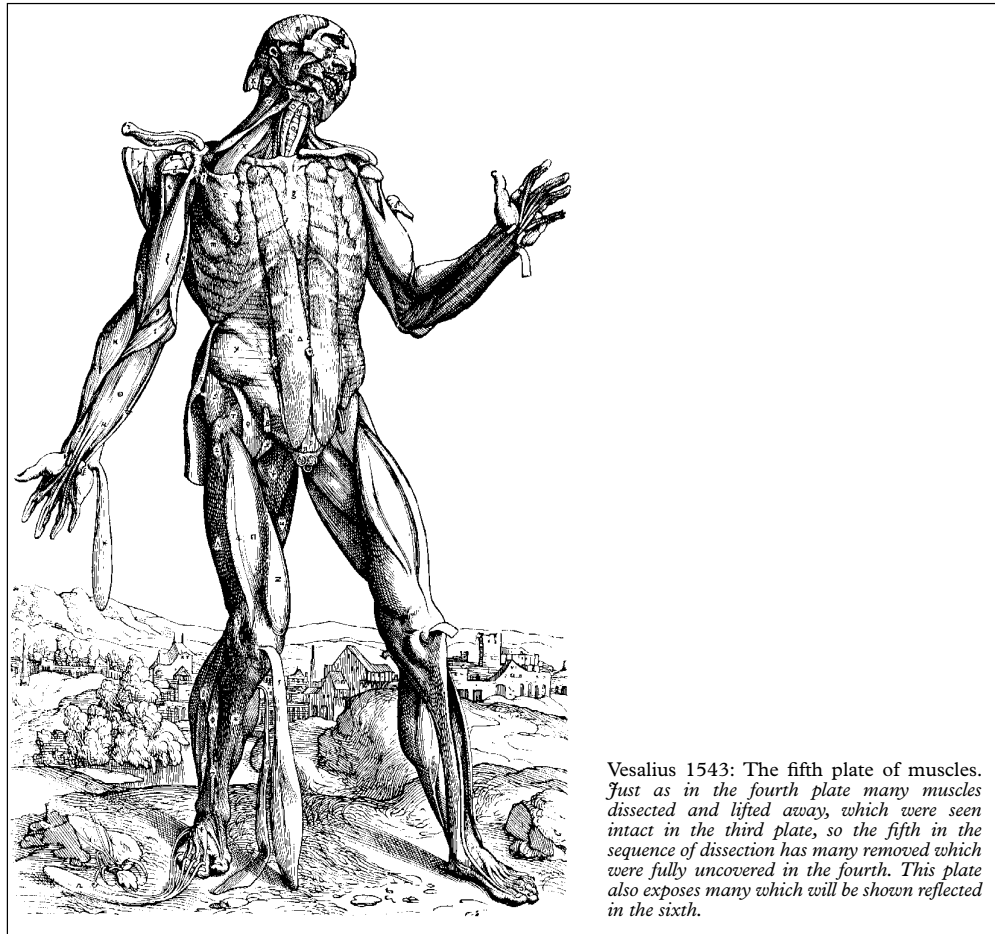
Taken together, these findings suggest the design of specific protocols using genetic markers in conjunction with aggressive therapeutics. These data, if they can be confirmed in other patient populations, suggest that typing RA patients to determine shared epitope status may be clinically useful. Larger studies to confirm these findings in other study populations are currently underway.

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Vesalius 1543: The fifth plate of muscles. Just as in the fourth plate many muscles dissected and lifted away, which were seen intact in the third plate, so the fifth in the sequence of dissection has many removed which were fully uncovered in the fourth. This plate also exposes many which will be shown reflected in the sixth.