HLA and Cytokine Gene Polymorphisms in Relation to Occurrence of Palindromic Rheumatism and Its Progression to Rheumatoid Arthritis

WALTER P. MAKSYMOWYCH, MARIA E. SUAREZ-ALMAZOR, HEIDI BUENVIAJE, BOBBI-LYNN COOPER, CAROLINE DEGEUS, MICHAEL THOMPSON, and ANTHONY S. RUSSELL

ABSTRACT. ***Objective.*** Palindromic rheumatism (PR) is an episodic arthropathy that may precede typical rheumatoid arthritis (RA), although pathogenetic relationships between these disorders remain unclear. The predictive value for those immunogenetic risk factors implicated in RA for disease progression in PR remains to be established. A previous retrospective analysis from our group has implicated rheumatoid factor in disease progression. Our objective was to determine the contribution of HLA and cytokine gene polymorphisms implicated in RA to predisposition to PR and to progression of PR to chronic joint inflammation.

Methods. We studied 147 patients with PR seen in a tertiary referral center; 87 were selected retrospectively from the period 1986–96 using a structured selection process and 60 were selected prospectively in the period 1997–2001. Comparison groups included 149 patients with RA and 149 ethnically matched controls. Typing for HLA-DRB1 alleles and HLA-DRB1-04 subtypes was performed following polymerase chain reaction (PCR) amplification using sequence-specific primers (SSP). Cytokine genotypes were ascertained following PCR-SSP with and without digestion with restriction enzymes. Time-adjusted survival analysis (Kaplan-Meier) and multivariate logistic regression were used to assess the independent contribution of immunogenetic markers in assessing progression of PR to chronic joint inflammation.

Results. Thirty-one percent of patients progressed to connective tissue disease after a mean of 10.6 (retrospective group) and 3.9 (prospective group) years. A significantly increased prevalence of the shared epitope (SE) allele was noted in patients with PR (65%) versus controls (39%) (OR 2.9, 95% CI 1.8–4.6, $p < 0.001$). This primarily reflected increased prevalence of the DRB1-0401 and 0404 and not DRB1-01 alleles. A weak contribution to disease susceptibility was also noted with carriage of the IL4 promoter –590T (OR 1.8, 95% CI 1.1–3.0, $p = 0.02$) and IL4 intron 3 RP1 (OR 1.7, 95% CI 1.1–2.9, $p = 0.03$) alleles. The TNFα +489A allele was associated with RA (OR = 2.7, 95% CI 1.5–5.1, $p = 0.001$) in both SE+ and SE– patients, but not with PR. Time-adjusted and multivariate Cox regression analysis revealed that only homozygosity for SE alleles was a significant independent risk factor for disease progression to chronicity in PR (hazard ratio 2.9, 95% CI 1.2–6.9, $p = 0.02$). However, none of 8 patients homozygous for SE– DRB1 XP4n alleles developed chronic disease after 10 years of followup ($p = 0.07$).

Conclusion. The immunogenetic risk profile for PR resembles that for RA, indicating that PR is likely not an independent entity. A significant gene dose effect for SE alleles is operative in determining risk for progression from PR to RA. (J Rheumatol 2002;29:2319–26)

Key Indexing Terms:
PALINDROMIC RHEUMATISM HLA GENES RHEUMATOID ARTHRITIS

From the Department of Medicine, University of Alberta, Edmonton, Alberta, Canada; and the Department of Medicine, Baylor College of Medicine, USA.

W.P. Maksymowych, FRCPC, Associate Professor; H. Buenviaje, Research Technician; B-L. Cooper, Research Technician; C. DeGeus-Wenceslau, MD, Rheumatologist; A.S. Russell, FRCPC, Professor, Department of Medicine, University of Alberta; M.E. Suarez-Almazor, MD, PhD, Associate Professor; M. Thompson, MS, Biostatistician, Department of Medicine, Baylor College of Medicine.

Address reprint requests to Dr. W.P. Maksymowych, 562 Heritage Medical Research Building, University of Alberta, Edmonton, Alberta T6G 2S2. E-mail: walter.maksymowych@ualberta.ca

Submitted January 3, 2002; revision accepted May 3, 2002.

Palindromic rheumatism (PR) was first described by Hench and Rosenberg in 1944[1] and is a disorder defined by the presence of sudden, recurrent, multiple, afebrile attacks of joint pain, swelling, and redness usually involving a single or a few joints. There is complete remission between episodes and physical findings, laboratory investigations, and radiographs are usually normal at the time of patient consultation. Attacks generally last a few hours to days and resolve without residual joint damage.

Several studies have shown that a significant number of patients subsequently develop another connective tissue

disease (CTD), the most common being rheumatoid arthritis (RA)[2-8]. Recently, a structured selection process was used to define a retrospective cohort of patients with PR from our center and showed that 34% had developed a CTD after a mean disease duration of 6 ± 6 years, RA being most common (28%)[8]. This was the first study to use time-adjusted analysis and multivariate survival methodology to assess the role of clinical prognostic factors. It identified female sex, positive rheumatoid factor (RF), and hand involvement as significantly increasing the risk for progression to RA. However, the role of immunogenetic factors implicated in RA disease susceptibility/severity was not examined.

Four studies have examined the role of HLA genes in susceptibility to PR and progression to RA[9-12]. These case control studies included small numbers of patients and did not follow a structured selection process, and typing for HLA antigens was serological. The results were inconsistent: 2 implicated HLA-DR4 in progression to RA and 2 did not.

There are at least 3 reasons why a more comprehensive analysis with larger numbers and using molecular DNA typing technology may be an important advance. First, there has been controversy whether PR is a separate entity or merely an incomplete expression of RA. Similarity in immunogenetic risk profile between PR and RA would favor the latter hypothesis. Second, there is continuing controversy whether immunogenetic associations with RA truly reflect disease susceptibility or, rather, disease severity as a consequence of selection bias. In particular, analysis of community based cohorts suggests little or no effect for the RA shared epitope (SE) in disease susceptibility, arguing for a role in determining disease severity[13,14]. These studies are hampered by the inclusion of patients who most likely do not have RA. PR appears to be prodromal for RA and characterization of the immunogenetic risk for progression to RA may also clarify the role of specific genes in RA disease susceptibility. Third, this analysis may be of practical clinical value in deciding which patients with PR are at highest risk for developing RA and therefore in need of closer supervision and perhaps more aggressive therapeutic intervention.

The association of the RA SE with disease in tertiary care settings is now well established, although there is continuing controversy as to the relative importance of different RA SE, specifically DRB1-04 subtypes, in relation to disease severity and specific phenotypic manifestations, e.g., nodule formation, extraarticular disease, requirement for surgery[15-18]. In addition, some studies[19,20], although not all[21,22], have highlighted the importance of homozygosity for the RA shared epitope and, in particular, DRB1-0401/0404 compound heterozygosity, in relation to disease severity. Several reports have also described a protective role for SE negative HLA-DR molecules that contain a negative or neutral electric charge in the P4 pocket (HLA-DRB1 XP4n) of the antigen-binding groove[23,24]. Others have used a targeted gene approach to examine polymorphisms in genes thought to play a role in the pathogenesis of RA. Several polymorphisms, including those in the interleukin 1, interleukin 4, and tumor necrosis factor-alpha cytokine gene loci, have recently been implicated in RA disease susceptibility/severity[25,26].

We examined HLA and cytokine polymorphisms previously implicated in RA in a cohort of patients with PR, selected prospectively as well as retrospectively, in comparison to local patients with RA attending a tertiary care facility. We also assessed potential associations with progression to RA using time-adjusted analysis and multivariate regression controlling for clinical risk factors identified in our earlier study[8].

MATERIALS AND METHODS

Patients. Caucasian patients with PR were selected from patients attending a university based outpatient rheumatology clinic. One group of patients was selected retrospectively following chart review of over 4900 patients with a diagnosis of arthritis seen from 1986 to 1996. The compilation of this cohort has been described[8]. Of 127 patients who complied with the inclusion criteria, 87 attended for clinical assessment and provided a blood sample. Sixty patients were also selected prospectively on a consecutive basis over the period 1997-2001. Patient inclusion criteria were developed on the basis of a review of the literature[27,28] and included (1) diagnosis of PR by a rheumatologist; (2) history of brief, sudden onset of recurrent episodes of mono- or oligoarthritis; and (3) at least 2 of the following criteria previously proposed: (i) direct observation of one attack by a physician, (ii) more than 5 attacks in 2 years, (iii) 3 or more joints involved in different attacks, (iv) normal radiographs, (v) reasonable exclusion of other recurrent monoarthritides such as gout, chondrocalcinosis or intermittent, periodic hydroarthrosis. Ethical approval was obtained from the University of Alberta Institutional Review Board.

The duration of PR was determined from the time of the first attack until the last clinical assessment if the patient did not develop another disease or until the development of RA or other CTD. The diagnosis of RA or other CTD was determined by a rheumatologist on the basis of American College of Rheumatology (ACR) criteria[29]. RF status was determined by nephelometry (positive titers ≥ 20 international units per ml).

An additional 149 Caucasian patients with RA attending the rheumatology outpatient clinic at the University of Alberta Hospital were invited to participate in the study on a consecutive basis. Patients fulfilled the ACR criteria for RA[29] and included 98 women and 51 men of mean age 61.3 years (range 33–74) and mean disease duration of 15.8 years (range 3–34). Controls included 149 unrelated healthy Caucasian individuals living in Edmonton, Alberta, of mean age 58.3 years (range 27–76).

DNA isolation and HLA-DR typing. Genomic DNA was isolated from anticoagulated peripheral blood using a modified salt precipitation method[30]. Low resolution typing of DRB1 genes was initially performed using sequence-specific primers and polymerase chain reaction (PCR) amplification (Fastype™ DR Low Resolution PCR-SSP Typing Kit, Biosynthesis Inc., Lewisville, TX, USA). For HLA-DRB1-04 positive individuals, a second amplification step was performed using primers specific for DRB1-04 subtypes (Fastype™ DRB1*04 PCR-SSP Subtyping Kit, Biosynthesis Inc.). Negative controls included samples without DNA.

Typing for cytokine allelic polymorphisms. Cytokine genotypes were determined as described[25] and required PCR for all the analyses. Negative controls included samples without DNA and positive controls included DNA samples where cytokine genotypes had been ascertained by DNA

sequencing. A polymorphism in the promoter region of IL4, representing a C-to-T substitution at position –590, was amplified and the PCR products studied after restriction digestion with *Bsm* FI (Table 1). Another IL4 polymorphism in the third intron is composed of a variable number of tandem repeats (VNTR) of a 70 bp sequence and was studied directly after amplification. A polymorphism in exon 5 of IL1ß was amplified and the PCR products studied after restriction digestion with *Taq*I. The polymorphism at position +489 in the first intron of the TNFα gene was studied after amplification using sequence-specific primers (forward primer 5'CGC TCT TCT GCC TGC TGC ACT T 3'; reverse primer 5' TCC CGC TCT TTC TGT CTC ACC A 3') for 35 cycles at 95°C for 30 s, 70°C for 30 s, and 72°C for 30 s. This was followed by digestion of PCR product with *Tai* I restriction enzyme. The TNFα +489A allele lacks the *Tai* I restriction site and is detected as a 420 bp product, while the TNFα +489G allele is detected as 246 and 174 bp products. All PCR products or digested fragments were examined following electropheresis on agarose gels stained with ethidium bromide.

Statistical analysis. Phenotypic frequencies were calculated and the chi-squared test, or Fisher's exact test where appropriate, were used to analyze differences between patients and controls. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated using logistic regression. Differences in survival times (free of RA or CTDs) were evaluated using Kaplan-Meier methods and log-rank tests. Patients who did not develop another disease were considered censored cases at the time of the last visit. Univariate and multivariate Cox regression models were used to identify variables associated with the development of RA and other CTDs. Hazard ratios were computed to estimate the risk for the variables of interest, including sex, RF, number of shared epitope alleles, number of HLA-DRB1 XP4n alleles, and specific cytokine gene phenotypes. Group status (prospective/retrospective) was also entered as a covariate in the COX analysis. The dependent variable was the duration of PR from the first attack until the development of another CTD or until the time of the last clinical assessment. All the analyses were conducted using SAS® and SPSS®. Statistical significance was set at 0.05. P values were not corrected since associations between the studied polymorphisms and RA have been described previously.

RESULTS

Table 2 shows selected demographic and clinical characteristics for the 2 groups of patients with PR. Mean disease duration is shown for both PR and the subsequent CTD and is, as expected, of longer duration in the retrospective as compared to the prospective cohort. Thirty-one percent progressed to a CTD, which was almost invariably RA, the others being systemic lupus (3 patients), and Behcet's disease (one patient). As expected, this was more commonly observed in the retrospective cohort.

Significant differences were observed in genotypic distributions of SE, TNFα +489, and DRB1 XP4n alleles, primarily in the RA population. Only SE genotypes significantly distinguished patients with PR from controls ($p < 0.001$). Homozygosity for SE alleles was noted in 14% of PR and 28% of RA patients versus 7% of controls. An increased prevalence of homozygosity for DRB1 XP4n negative alleles was evident in the RA (63%) and PR (58%) populations in comparison to controls (48%), although only the former comparison was statistically significant ($p = 0.03$).

Table 3 shows the phenotypic frequencies for HLA and cytokine polymorphisms in populations of patients with PR and RA in comparison to controls. There was a significantly increased risk for development of both PR and RA in those patients with at least one SE allele. Homozygosity for the SE further increased the risk for disease in the RA population (OR 10.7, 95% CI 4.8–23.6), but much less so in those with PR (OR 3.5, 95% CI 1.5–8.0). The presence of a DRB1 XP4n allele significantly decreased the risk for development of RA, although the reduced risk noted in patients with PR did not attain statistical significance. Homozygosity for DRB1 XP4n alleles further reduced the risk for disease, although numbers were small (for PR OR 0.6, 95% CI 0.2–1.4; for RA OR 0.4, 95% CI 0.2–1.2). Examination of cytokine polymorphisms showed a weak although significant contribution to disease susceptibility from carriage of

Table 1. Cytokine polymorphisms and techniques used for analysis.

	IL1β Exon 5	IL4 Intron 3	IL4 Promoter	TNFα + 489
Type of polymorphism	Single base	70 bp VNTR	Single base C/T	Single base A/G
Site of polymorphism	Exon 5	Intron 3	–590	+489
PCR primers				
Upstream	5′GTTGTCATCAG-ACTTTGACC-3′	5′AGGCTGAAAG-GGGGAAAGC-3′	5′ACTAGGCCTCA-CCTGATACG-3′	5′CGCTCTTCTGC-CTGCTGCACTT-3′
Downstream	5′TTCAGTTCATA-TGGACCAGA-3′	5′CTGTTCACCTC-AACTGCTCC-3′	5′GTTGTAATGCA-GTCCTCCTG-3′	5′TCCCGCTCTTT-CTGTCTCACCA-3′
PCR conditions				
Denaturation	95°C 30 s	95°C 30 s	95°C 30 s	95°C 30 s
Annealing	62°C 20 s	66°C 20 s	66°C 20 s	70°C 30 s
Extension	72°C 20 s	72°C 20 s	72°C 20 s	72°C 30 s
No. of cycles	30	30	30	35
Digestion	Yes (*Taq* I)	No	Yes (*Bsm* FI)	Yes (*Tai* I)
Allele size, bp	E1:135 + 114 E2:249	RP1:183 RP2:253	IL4 –590C:192 + 60 IL4 –590T:252	TNFα + 489G:246 + 174 TNFα + 489A:420

VNTR: variable number of tandem repeats.

Table 2. Selected demographic and clinical features of 147 patients with palindromic rheumatism.

	Retrospective Cohort, n = 87	Prospective Cohort, n = 60	p
Females, n (%)	66 (76)	39 (65)	NS
Mean age (SD), yrs	50.8 (11.0)	44 (10.8)	NS
Mean disease duration (SD), yrs			
Palindromic rheumatism	10.6 (7.8)	3.9 (5.6)	0.002
Connective tissue disease	5.6 (5.4)	0.8 (0.6)	0.02
Rheumatoid factor* (%) (n = 135)	37 (46)	21 (38)	NS
Development of RA or other CTD (%)			
RA	29 (33)	12 (20)	0.04
Other**	4 (5)	0	

* At diagnosis of palindromic rheumatism. ** One had Behcet's disease, 3 had SLE.

Table 3. Phenotypic frequencies of HLA and cytokine polymorphisms in patients with palindromic rheumatism (PR), rheumatoid arthritis (RA), and controls.

Polymorphism	Controls, n = 149 (%)	PR, n = 147 (%)	OR (95% CI)	p*	RA, n = 149 (%)	OR (95%CI)	p*
SE+	58 (39)	95 (65)	2.9 (1.8–4.6)	< 0.001	114 (77)	5.1 (3.1–8.4)	< 0.001
DRB1 XP4n+	78 (52)	62 (42)	0.7 (0.4–1.0)	0.08	55 (37)	0.5 (0.3–0.9)	0.008
IL4 Promoter							
–590T	35 (23)	53 (36)	1.8 (1.1–3.0)	0.02	52 (35)	1.7 (1.1–2.9)	0.03
–590C	145 (97)	139 (95)	0.5 (0.1–1.6)	NS	140 (94)	0.4 (0.1–1.4)	NS
IL4 intron 3							
RP1	36 (24)	53 (36)	1.8 (1.1–2.9)	0.03	51 (34)	1.6 (1.0–2.7)	0.06
RP2	146 (98)	138 (94)	0.3 (0.1–1.2)	0.09	140 (94)	0.3 (0.1–1.2)	0.09
IL1β exon 5							
E1	57 (38)	52 (35)	0.9 (0.6–1.4)	NS	58 (39)	1.0 (0.6–1.6)	NS
E2	145 (97)	140 (95)	0.6 (0.2–1.9)	NS	143 (96)	0.7 (0.2–2.4)	NS
TNFα + 489							
A	18 (14)	24 (16)	1.4 (0.7–2.7)	NS	41 (28)	2.7 (1.5–5.1)	0.001
G	144 (97)	144 (98)	1.7 (0.4–7.1)	NS	142 (95)	0.7 (0.2–2.3)	NS

SE: shared epitope allele; XP4n: negative P4 charge in HLA-DRB1 antigen-binding pocket; NS: not significant; *Comparisons with controls

IL4 promoter –590T and IL4 intron 3 RP1 alleles in both patient populations. However, these alleles were noted to be in strong linkage disequilibrium (data not shown). Simultaneous carriage of these alleles was noted in 23.5% of controls compared to 33.3% (p = 0.06) and 34.2% (p = 0.04) of patients with PR and RA, respectively. A significantly increased risk for disease in those carrying the TNFα +489A allele was only noted in the RA population. This was evident in both SE+ and SE– patients (Table 4). Phenotypic frequencies of the TNFα +489A allele in SE+ and SE– patients with PR were intermediate between controls and patients with RA.

Comparisons of HLA-DRB1 genotypes previously associated with RA showed a significantly increased risk for both PR and RA in those carrying the DRB1-0401 and 0404 alleles (Table 5). Genotypes DRBI-0401/0404 and DRB1-01/0401 were only observed in the patient populations. There were no significant differences in carriage of DRB1-01, even in DRB1-04 negative individuals (data not shown).

Comparison of HLA and cytokine genotypic frequencies in patients with PR according to the development of CTD, before adjusting for period of observation, revealed statistically significant differences in genotypic distribution only for SE alleles (p = 0.04). Figure 1 shows the cumulative survival curve for SE and DRB1 XP4n alleles. By 7 years, at least 50% of patients with PR who were homozygous for SE alleles had developed CTD (p = 0.009). The presence of one SE allele alone did not influence progression, regardless of whether this was DRB1-0401 or 0404 (data not shown). Median duration from onset of PR until the development of RA was 7 years (range 5–17) in those carrying 2 SE alleles compared to 18 (range 12–30) and 21 years (range 12–35) in those carrying one and zero SE alleles, respectively. A borderline statistical association was observed between DRB1 XP4n alleles and disease progression (p = 0.07). Progression was not observed in any of 8 patients that were homozygous for DRB1 XP4n alleles after 10 years of followup. Examination of cytokine polymorphisms using survival analysis revealed no significant effects on disease

Table 4. Phenotypic frequencies of TNFα + 489A allele in the presence or absence of the shared epitope (SE) allele in patients with palindromic rheumatism (PR), rheumatoid arthritis, and controls.

	Controls, n = 149 (%)	PR, n = 147 (%)	OR (95%CI)	p*	RA, n = 149 (%)	OR (95% CI)	p*
SE+	3/58 (5)	11/95 (12)	2.4 (0.64–9.0)	NS	25/114 (22)	5.1 (1.5–17.9)	0.01
SE–	15/91 (16)	13/52 (25)	1.7 (0.73–3.9)	NS	15/35 (43)	3.8 (1.6–9.1)	0.003

*Comparisons with controls.

Table 5. HLA-DRB1 genotypes observed in patients with palindromic rheumatism (PR), rheumatoid arthritis, (RA) and controls.

HLA-DRB1 Combinations	Controls n	(%)	PR, n	(%)	OR (95% CI)	p*	RA, n	(%)	OR (95% CI)	p*
0401/X	15	(11)	39	(27)	3.2 (1.7–6.2)	< 0.001	51	(34)	4.6 (2.5–8.7)	< 0.001
0404/X	10	(7)	22	(15)	2.4 (1.1–5.4)	0.03	28	(19)	3.2 (1.5–6.9)	< 0.001
0401/0401	3	(2)	6	(4)	2.1 (0.51–8.4)	NS	9	(6)	3.1 (0.83–11.8)	0.09
0401/0404	0	(0)	2	(1)	**	NS	9	(6)	**	0.003
0401/DRB1 01	0	(0)	5	(3)	**	0.03	11	(7)	**	0.001
DRB1 01	33	(22)	27	(18)	0.79 (0.45–1.4)	NS	44	(30)	1.5 (0.87–2.5)	NS

* Comparisons with controls.**OR cannot be computed (zero cells), statistical significance estimated with Fisher's exact test. X: alternative HLA allele.

progression (data not shown). Furthermore, we did not observe significant interactions between cytokine polymorphisms and SE alleles on disease progression (data not shown). The presence of RF significantly decreased time to disease progression (p = 0.04), seropositive patients progressing after a median of 12 years (9–15) compared to 30 years (15–35) for seronegative patients. However, no significant interactions with SE alleles were evident (data not shown).

Table 6 shows the results of the Cox regression models (univariate and multivariate). After including age, female sex, and RF, the risk associated with homozygosity for SE alleles remained statistically significant, the risk for developing CTD being 2.9. Homozygosity for DRB1 XP4n alleles could not be included in this analysis because none of these individuals had progressed by 10 years. Analysis of cytokine polymorphism phenotypes revealed no independent effects on risk for disease progression (data not shown). No significant additive interactions were noted in the analysis of SE alleles and RF, and SE alleles and cytokine polymorphisms in the risk for progression (data not shown). In addition, no significant differences were observed between patients with PR selected retrospectively or prospectively when group was entered as a covariate in the Cox analysis.

DISCUSSION

Our findings highlight the risk conveyed by homozygosity for SE alleles in susceptibility to PR as well as progression to RA. In addition, this effect is independent of clinical and other immunogenetic markers previously implicated in RA susceptibility and severity. In contrast, homozygosity for shared epitope negative HLA-DRB1 alleles appears to be protective with respect to disease progression. Cytokine polymorphisms were associated with weak effects on disease susceptibility in both patient populations and did not influence disease progression in patients with PR.

Several studies have examined the role of HLA genes in predisposition to PR and progression to RA[9-12]. Some, but not all, reported associations with HLA-DR4. This lack of consistency is most likely due to small numbers, variable duration of followup, and differences in sampling procedures. Our study is the first to use time-adjusted analysis to address differences in duration of followup and multivariate regression to examine the role of confounding factors, and to include patients selected prospectively in an analysis of immunogenetic risk factors for both susceptibility and progression to RA. Finally, we included an ethnically matched cohort of patients with well established RA to allow for further comparisons of genotypic and phenotypic frequencies of the targeted genes.

It is apparent that the major immunogenetic risk factor among those genes examined is the shared epitope, particularly 2 copies. We observed a hierarchical risk among SE alleles, there being no contribution from the DRB1-01 SE allele as noted in previous studies of RA populations[30,31]. There may also be a protective effect associated with homozygosity for SE negative HLA-DRB1 alleles that have a neutral or negative P4 pocket in the class II antigen-binding groove. Of 8 such patients, none have developed RA after 10 years of PR. A previous study of 3 different French populations described protective effects of DRB1 XP4n alleles independent of carriage of SE alleles[24]. This will require confirmation after further followup and with

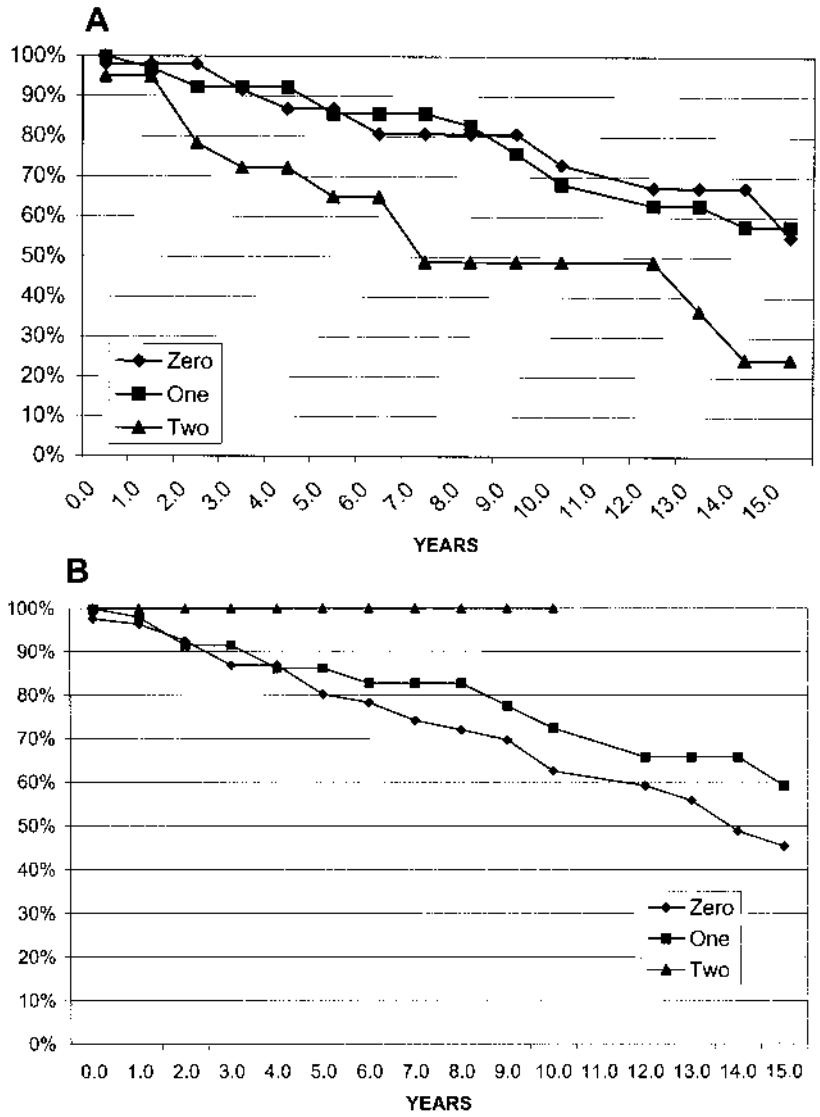

Figure 1. Survival analysis (Kaplan-Meier) for progression to connective tissue disease in patients with palindromic rheumatism according to (A) number of SE alleles, (B) number of DRB1 XP4n alleles.

larger patient numbers. These HLA locus observations in PR were also noted in the RA cohort, arguing for a SE dose effect in susceptibility to both PR and RA. Further, the risk for progression to RA in those homozygous for SE alleles was independent of RF status. Our findings are consistent with a recent analysis of prognostic factors for structural damage in early RA. Of several clinical and laboratory markers examined, logistic regression analysis revealed that the only baseline values predictive of 3 year radiologic scores were RF positivity, DRB1-04 genes, erythrocyte sedimentation rate, and erosion score at baseline[32]. As well, patients homozygous for DRB1-04 alleles had the worst outcome, consistent with reports suggesting a gene dosage effect in RA severity[33,34]. On the other hand, other reports indicate an effect of SE alleles in predicting radiographic outcome only in RF– but not RF+ patients with RA in both early as well as longstanding RA, and no gene dosage effect[35,36]. These discrepancies may reflect differences in patient selection and/or different radiographic outcome measures. It has been suggested that early and aggressive disease modifying antirheumatic drug therapy affects the association of HLA class II alleles with progression of joint damage in RA[37]. We previously reported that most of our patients with PR are treated with antimalarials, so that this consideration seems an unlikely explanation for the prognostic influence of SE alleles[38]. Our study lacked sufficient numbers to examine the risks associated with specific combinations of SE alleles.

Examination of cytokine gene polymorphisms revealed weak contributions from polymorphisms in the IL4

Table 6. Univariate and multivariate Cox regression models (age-adjusted) for progression of palindromic rheumatism to chronic connective tissue disease.

	Univariate Analysis			Multivariate Analysis		
	Hazard Ratio	(95% CI)	p	Hazard Ratio	(95% CI)	p
Female sex	0.9	(0.5–1.8)	NS	1.0	(0.5–2.1)	NS
RF	1.9	(1.0–3.7)	0.04	1.9	[1.0–3.7]	0.06
SE–/SE+	1.1	[0.6–2.3]	NS	1.2	[0.6–2.6]	NS
SE+/SE+	3.0	[1.3–6.6]	0.008	2.9	[1.2–6.9]	0.02
DRB1 XPn4						
–/+	0.8	[0.4–1.5]	NS	1.0	[0.5–2.1]	NS
+/+	*			*		

*Could not be included in the Cox regression analysis because no DRB1 XP4n homozygote developed disease.

promoter and intron 3 regions to susceptibility for both PR and RA. This is consistent with findings in a report that also revealed an even greater risk from simultaneous carriage of the IL4 –590T promoter and IL4 RP1 alleles in patients with RA compared to controls[25]. These alleles were in strong linkage disequilibrium in our control population and simultaneous carriage was not, therefore, associated with any further increases in disease susceptibility in the 2 patient populations. A report has also suggested additive interactions between polymorphism in exon 5 of the IL1ß gene and SE alleles in disease severity and development of joint erosions in RA[25]. However, our analysis did not reveal such an interaction in patients with PR that progressed to RA, suggesting that this association may primarily reflect pathophysiological mechanisms leading to joint erosions rather than chronic inflammation per se.

Increased phenotypic frequency of the TNFα +489A allele was noted in RA independently of SE alleles. This was not evident in patients with PR, nor was there a significant contributory role in disease progression either independently or in the presence of SE alleles. This suggests that the association might reflect disease severity rather than susceptibility. These data contrast with a study that described a protective role for the TNFα +489A allele in the development of joint erosions in RA[26]. This polymorphism is not associated with any particular combination of HLA alleles and is not a marker of conserved ancestral HLA haplotypes, although there is linkage disequilibrium between TNFα +489A and HLA-DR14, B8, and the TNFα10 microsatellite[39]. Further, this polymorphism does not appear to be associated with changes in TNFα transcriptional activity[40]. Consequently, the association noted in this study requires further confirmation.

This study represents the largest cohort of patients with PR, and the immunogenetic similarities with a local cohort of patients with RA point to this disorder being an incomplete expression of RA. There are, however, some limitations to our study. About half of our study population was retrospectively selected and all patients attended a tertiary referral center, so that the associations described here may be less evident in cases diagnosed in the community. The lack of a well defined case definition also allows the inclusion of patients with other rheumatic disorders. Confirmation of our observations will require further analysis of patients selected prospectively and longer duration of followup.

Our study has shown that the presence of a shared epitope allele and, in particular, homozygosity for SE alleles is associated with susceptibility to both PR and RA. Further, homozygosity for SE alleles significantly increases the risk for progression to RA, independent of RF status. Conversely, homozygosity for SE negative DRB1 XP4n alleles may be protective. Finally, polymorphisms within the IL4 gene provide a weak contribution to disease risk in both patient populations, but polymorphisms in the IL1ß and TNFα genes previously implicated in RA do not appear to influence susceptibility to PR or progression to RA.

REFERENCES

1. Hench JJ, Rosenberg EF. Palindromic rheumatism. Arch Intern Med 1994;73:293-321.
2. Hannonen P, Mottonen T, Oka M. Palindromic rheumatism, a clinical survey of sixty patients. Scand J Rheumatol 1987; 16:413-20.
3. Mattingly S. Palindromic rheumatism. Ann Rheum Dis 1966;25:307-17.
4. Williams MH, Sheldon PJHS, Torrigiani G, Eisen V, Mattingly S. Palindromic rheumatism. Clinical and immunological studies. Ann Rheum Dis 1971;30:375-80.
5. Wajed MA, Brown DL, Currey HLF. Palindromic rheumatism. Clinical and serum complement study. Ann Rheum Dis 1977; 36:56-61.
6. Eliakim A, Neumann L, Horowitz J, Buskila D. Kleiner-Baumgarten A, Sukenik S. Palindromic rheumatism in Israel: a disease entity? A survey of 34 patients. Clin Rheumatol 1989;8:507-11.
7. Youssef WA, Yan A, Russell AS. Palindromic rheumatism: A response to chloroquine. J Rheumatol 1991;18:35-7.
8. Gonzalez-Lopez L, Gamez-Nava JI, Jhangri GS, Ramos-Remus C, Russell AS, Suarez-Almazor ME. Prognostic factors for the development of rheumatoid arthritis and other connective tissue diseases in patients with palindromic rheumatism. J Rheumatol

1999;26:540-5.
9. Bregeon C, Dajon JL, Renier G, et al. Palindromic rheumatism. Immunologic survey and study of development in 43 cases. Rev Rhum Mal Osteoartic 1986;53:441-9.
10. Barbieri P, Ciompi ML, Menicucci A, Pasero G. HLA antigens in palindromic rheumatism. An Italian study. Clin Rheumatol 1988;7:470-3.
11. Fisher LR, Kirk A, Awad J, et al. HLA antigens in palindromic rheumatism and palindromic onset rheumatoid arthritis. Br J Rheumatol 1986;25:345-8.
12. Gran JT, Husby G, Thorsby E. HLA antigens in palindromic rheumatism, nonerosive rheumatoid arthritis and classical rheumatoid arthritis. J Rheumatol 1984;11:136-40.
13. Thomson W, Harrison B, Ollier B, et al. Quantifying the exact role of HLA-DRB1 alleles in susceptibility to inflammatory polyarthritis. Arthritis Rheum 1999;42:757-62.
14. De Jongh BM, van Romunde LKJ, Valkenburg HA, de Lange GG. Epidemiological study of HLA and GM in rheumatoid arthritis and related symptoms in an open Dutch population. Ann Rheum Dis 1984;43:613-9.
15. Weyand CM, Xie C, Goronzy JJ. Homozygosity for the HLA-DRB1 allele selects for extraarticular manifestations in rheumatoid arthritis. J Clin Invest 1992;89:2033-9.
16. Hall FC, Weeks DE, Camilleri JP, et al. Influence of the HLA-DRB1 locus on susceptibility and severity in rheumatoid arthritis. Q J Med 1996;89:821-9.
17. Meyer JM, Evans TI, Small RE, et al. HLA-DRB1 genotype influences risk for and severity of rheumatoid arthritis. J Rheumatol 1999;26:1024-34.
18. Weyand CM, McCarthy TG, Goronzy JJ. Correlation between disease phenotype and genetic heterogeneity in rheumatoid arthritis. J Clin Invest 1995;95:2120-6.
19. Wordsworth P, Pile K, Buckely J, et al. HLA heterozygosity contributes to susceptibility to rheumatoid arthritis. Am J Hum Genet 1992;51:585-91.
20. Jawaheer D, Thomson W, MacGregor AJ, et al. "Homozygosity" for the HLA-DR shared epitope contributes the highest risk for rheumatoid arthritis concordance in identical twins. Arthritis Rheum 1994;37:681-6.
21. Rau R, Herborn G, Zueger S, Fenner H. The effect of HLA-DRB1 genes, rheumatoid factor, and treatment on radiographic disease progression in rheumatoid arthritis over 6 years. J Rheumatol 2000;27:2566-75.
22. Suarez-Almazor ME, Tao S, Moustarah F, Russell AS, Maksymowych W. HLA-DR1, DR4 and DRB1 disease related subtypes in rheumatoid arthritis: association with susceptibility but not severity in a city wide community based study. J Rheumatol 1995;22:2027-37.
23. Vos K, van der Horst-Bruinsma IE, Hazes JMW, et al. Evidence for a protective role of the human leukocyte antigen class II region in early rheumatoid arthritis. Rheumatology 2001;40:133-9.
24. Reviron D, Perdriger A, Toussirot E, et al. Influence of shared epitope-negative HLA-DRB1 alleles on genetic susceptibility to rheumatoid arthritis. Arthritis Rheum 2001;44:535-40.
25. Cantagrel A, Navaux F, Loubet-Lescoulie P, et al. Interleukin-1ß, interleukin-1 receptor antagonist, interleukin-4, and interleukin-10 gene polymorphisms: Relation to occurrence and severity of rheumatoid arthritis. Arthritis Rheum 1999;42:1093-100.
26. van Krugten MV, Huizinga TW, Kaijzel EL, et al. Association of the TNF +489 polymorphism with susceptibility and radiographic damage in rheumatoid arthritis. Genes Immunol 1999;1:91-6.
27. Pasero G, Barbieri P. Palindromic rheumatism: you just have to think about it! Clin Exp Rheumatol 1986;4:197-9.
28. Guerne PA, Weisman MH. Palindromic rheumatism: part or apart from the spectrum of rheumatoid arthritis. Am J Med 1992; 93:451-60.
29. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315-24.
30. Van Jaarsveld CHM, Otten HG, Jacobs JWG, Kruize AA, Brus HLM, Bijlsma JWJ. Association of HLA-DR with susceptibility to and clinical expression of rheumatoid arthritis: re-evaluation by means of genomic tissue typing. Br J Rheumatol 1998;37:411-6.
31. Rønningen KS, Spurkland A, Egeland T, et al. Rheumatoid arthritis may be primarily associated with HLA-DR4 molecules sharing a particular sequence at residue 67-74. Tissue Antigens 1990; 36:236-40.
32. Combe B, Dougados M, Goupille P, et al. Prognostic factors for radiographic damage in early rheumatoid arthritis. Arthritis Rheum 2001;44:1736-43.
33. Gough A, Faint J, Salmon M, et al. Genetic typing of patients with inflammatory arthritis at presentation can be used to predict outcome. Arthritis Rheum 1994;37:1166-70.
34. El-Gabalawy HS, Goldbach-Mansky R, Smith D II, et al. Association of HLA alleles and clinical features in patients with synovitis of recent onset. Arthritis Rheum 1999;42:1696-705.
35. Harrison B, Thomson W, Symmons D, et al. The influence of HLA-DRB1 alleles and rheumatoid factor on disease outcome in an inception cohort of patients with early inflammatory arthritis. Arthritis Rheum 1999;42:2174-83.
36. Mattey DL, Hassell AB, Dawes PT, et al. Independent association of rheumatoid factor and the HLA-DRB1 shared epitope with radiographic outcome in rheumatoid arthritis. Arthritis Rheum 2001;44:1529-33.
37. Lard LR, Boers M, Verhoeven A, et al. Early and aggressive treatment of rheumatoid arthritis patients affects the association of HLA class II antigens with progression of joint damage. Arthritis Rheum 2002;46:899-905.
38. Avina-Zubieta JA, Galindo-Rodriguez G, Newman S, Suarez-Almazor ME, Russell AS. Long-term effectiveness of antimalarial drugs in rheumatic diseases. Ann Rheum Dis 1998;57:582-7.
39. D'Alfonso S, Momigliano RP. An intragenic polymorphism in the human tumor necrosis factor alpha (TNFA) chain-encoding gene in Japanese. Immunogenetics 1996;44:321-2.
40. Verweij CL. Tumour necrosis factor gene polymorphisms as severity markers in rheumatoid arthritis. Ann Rheum Dis 1999;58 Suppl 1:120-6.