Familial Seropositive Rheumatoid Arthritis in North American Native Families: Effects of Shared Epitope and Cytokine Genotypes

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ABSTRACT. ***Objective.*** A number of North American native (NAN) populations have high prevalence rates of both rheumatoid arthritis (RA) and the shared epitope (SE). We examined the phenotype and familial incidence of RA in a NAN population, and investigated how the SE and cytokine genes may affect disease risk within affected families.

Methods. NAN patients with seropositive RA or polyarthritis rheumatoid factor (RF) positive juvenile idiopathic arthritis (JIA) were identified from clinical databases. Patients were recruited consecutively as they presented for clinic visits. Family pedigrees were constructed and consenting relatives were interviewed and examined. The risk of RA within families was calculated by multiple logistic regression. Input variables were the SE and cytokine genotypes. Probands and affected relatives were entered as the affected group, and unaffected relatives within families as the unaffected group. Results were confirmed among unrelated subjects, i.e., unrelated patients and unaffected relatives of other probands.

Results. The familial prevalence of RA was 0.50 (95% confidence intervals 0.30, 0.70) among 28 families studied. The interleukin 10 (IL-10) promoter –1082G/A genotype decreased the odds of RA relative to the A/A genotype in affected families (OR 0.247, 95% CI 0.081, 0.751; p = 0.014) and among unrelated subjects (OR 0.203, 95% CI 0.064, 0.640; p = 0.006). The G/G genotype yielded an OR of 0.093 (95% 0.013, 0.676; p = 0.019) among unrelated subjects. The SE had no effect in these calculations.

Conclusion. There was a high familial prevalence of RA in this NAN cohort. In susceptible NAN families, the risk of RA was reduced by IL-10 genotypes, whereas the SE did not affect risk. Study of healthy NAN controls is required to determine if these conclusions apply to this NAN population as a whole. (J Rheumatol 2005;32:983–91)

Key Indexing Terms:
RHEUMATOID ARTHRITIS
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Based on concordance rates in monozygotic twins in Caucasian populations, the maximum genetic contribution to rheumatoid arthritis (RA) has been estimated at 12% to 15%[1,2], although more recent analyses indicate that genetic factors account for roughly 60% of the variation in disease liability[3]. However, HLA accounts for only an estimated one-third of the genetic component[1,4,5]. The candidate gene approach is an attractive method to identify genes, which may explain the remaining genetic susceptibility[2]. Among such candidate genes are single nucleotide polymorphisms (SNP) in genes involved in the inflammatory response. To date, several associations of RA with cytokine gene polymorphisms have been described[6-14]. However, many of these associations are inconsistent between populations and/or have had low odds ratios[15-19]. Several factors may account for this, including modest effects of individual genes in comparison to the shared epitope (SE), ethnic differences in allele frequencies, and population stratification. North American Natives (NAN) are a useful population for genetic studies in RA. Archeological evidence suggests a NAN origin for RA[20,21]. In addition, an increased prevalence, younger age of onset, and increased severity of RA in NAN suggest a greater genetic susceptibility than in non-

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NAN populations[20,22-29]. Ethnic homogeneity within tribal groups may also reduce the risk of false-positive associations due to population stratification.

We have reported a high prevalence of the SE (59%) in NAN in Manitoba and Northwestern Ontario[30]. While the odds of RA are increased nearly 5-fold by the SE in this population[31], the high frequency of the SE itself suggests there are additional genes that increase risk in those affected, or protective genes in those unaffected. Assuming a high genetic component for RA in this population, affected families would be expected to have an even higher concentration of non-SE RA-promoting genes. Further, it can be expected that protective genes would be more easily detected in unaffected family members of probands than in the population at large, particularly those who are beyond the usual age of onset of RA observed in this population. The use of family members as controls minimizes population stratification even further.

We describe the clinical features of RA in NAN patients seen in a rheumatology specialty center, present an analysis of familial cases, and analyze the effect of the SE and several cytokine polymorphisms on the risk of RA in affected families.

MATERIALS AND METHODS

Patients and relatives. Patients were identified from prospectively collected patient registries kept at the University of Manitoba Arthritis Centre and the Children's Hospital Pediatric Rheumatology Clinic, Winnipeg. Criteria for selection were NAN descent, a diagnosis of RA or polyarthritis rheumatoid factor (RF) positive juvenile idiopathic arthritis (JIA) by American College of Rheumatology (ACR) and International League of Associations for Rheumatology (ILAR) criteria, respectively[32,33], positive RF, and a disease duration of at least 2 years. A person of NAN descent was defined as one with 2 or more NAN grandparents. Patients were recruited sequentially as they presented for scheduled followup appointments in the rheumatology clinics at the University of Manitoba Arthritis Centre or Children's Hospital, Winnipeg. The medical charts of consenting patients were reviewed for dates of onset and first visit to our clinics, results of RF and antinuclear antibody (ANA) tests, use of disease modifying antirheumatic drugs (DMARD), surgical reports, and presence of erosions and joint space loss on reports of radiographs. RF was determined in the hospital laboratories by routine nephelometry. A value > 20 international units is considered positive. ANA was detected using HEp-2 substrates, and a titer > 1:40 is considered positive. Patients were interviewed by the study nurse and were examined by rheumatologists. Patients and affected relatives completed a Health Assessment Questionnaire (HAQ)[34].

Patients with polyarthritis RF-positive JIA were included as this condition can be considered to be an early onset of seropositive RA clinically.

Family pedigrees were constructed with the help of probands. Consenting family members were interviewed and examined to confirm or eliminate a diagnosis of RA. Where affected family members were also attending our clinics, the patient recruited first served as the proband. Only those relatives confirmed as having RA by examination and interview were included as affected.

The project was approved by the University of Manitoba Biomedical Research Ethics Board. Written informed consent was obtained from all participants.

HLA typing. DRB1 typing was performed by polymerase chain reaction (PCR) using sequence-specific primers and probes. Frequencies of the SE were based on the presence of DRB1*0101, 0401, 0404, 0408, 1410, and 1402, SE+ alleles previously found in this population[30].

Cytokine gene alleles. Tumor necrosis factor-α (TNF-α), transforming growth factor-ß (TGF-ß), interleukin 10 (IL-10), IL-6, and interferon-γ (IFN-γ) genes were studied, as these cytokines are either proinflammatory (TNF, IL-6, IFN-γ) or have immunosuppressive and antiinflammatory activity (IL-10, TGF-ß)[35]. Further, polymorphisms of each have been associated with differentials in gene transcription or *in vitro* protein synthesis, and have been found to affect the risk or severity of RA in studies of other populations[6-12,36-46]. Cytokine gene allele typing was performed by polymerase chain reaction with sequence-specific primers for the following alleles: TNF-α promoter –308G and A (rs number 1800629), TGF-ß codon 10T and C (rs number 1982073), TGF-ß codon 25G and C (rs number 1800471), IL-10 promoter –1082G and A (rs number 1800896), IL-10 promoter –819C and T (rs number 1800871) and IL-10 promoter –592A and C (rs number 1800872), IL-6 promoter –174 G and C (rs number 1800795), and IFN-γ intron 874T and A (rs number 3138557)[47] (One Lambda Inc., Canoga Park, CA, USA). IL-10 genotypes are listed separately for each polymorphism, as haplotypes could not be proved. However, homozygous typings suggested the following haplotypes: IL-10 promoter –1082G –819C –592C (GCC), ATA, and ACC, similar to those in other populations[40,41].

Calculations. Allele frequencies were calculated according to the formula[48]:

$$p = f_{A/A} + 1/2f_{A/a} = \text{frequency of A allele}$$
$$q = 1 - p = \text{frequency of a allele}$$

Comparisons between groups were made by paired t tests, Mann-Whitney U, chi-square, or Fischer exact tests.

Risks for RA were estimated by calculating odds ratios (OR) in multiple regression analyses. Analyses were performed by multiple logistic regressions, entering variables by both forward and backward conditional methods. Possible explanatory variables entered were the SE and each of the 3 possible genotypes for each cytokine gene polymorphism. The homozygous low-producing genotype was entered as reference. Statistical analyses were performed using SPSS, version 10 (SPSS Inc., Chicago, IL, USA) and SAS, version 8.2 (SAS Institute Inc., Cary, NC, USA). The level of significance was set at $p = 0.05$.

Since the objective was to detect protective genes in unaffected relatives of probands, analyses were performed first within the 28 families studied. Probands and affected relatives were entered as the affected group (RA group A1) and unaffected relatives as the unaffected group (family control group A2; Figure 1). Both nonstratified logistic regression and a conditional logit analysis, stratifying within each family, were performed. In a second analysis, patients whose families were not studied and patients with no unaffected relatives in the study were entered as the affected group (RA group B1), and unaffected relatives of other probands were entered as the unaffected group (family control group B2; Figure 1). For families in which there was more than one unaffected member, only the elder subject was entered. Thus neither patients nor unaffected relatives were related to each other within or between the groups.

In all 28 probands, 18 affected and 27 unaffected relatives and 54 patients without family studies were recruited. HLA typing was missing for 2 affected relatives and one patient with no family study; and cytokine genotyping was missing for 2 affected relatives and one proband.

RESULTS

Demographic and clinical characteristics. Eighty-two of 169 NAN patients meeting the selection criteria and 45 relatives participated in the study. Eighty percent of patients had 4, 16% had 3, and 2% had 2 NAN grandparents, indicating relatively homogeneous ethnic origins for these patients. All were Cree or Ojibway, except for 4 who were Siouan and one who was Dene. The clinical and demo-

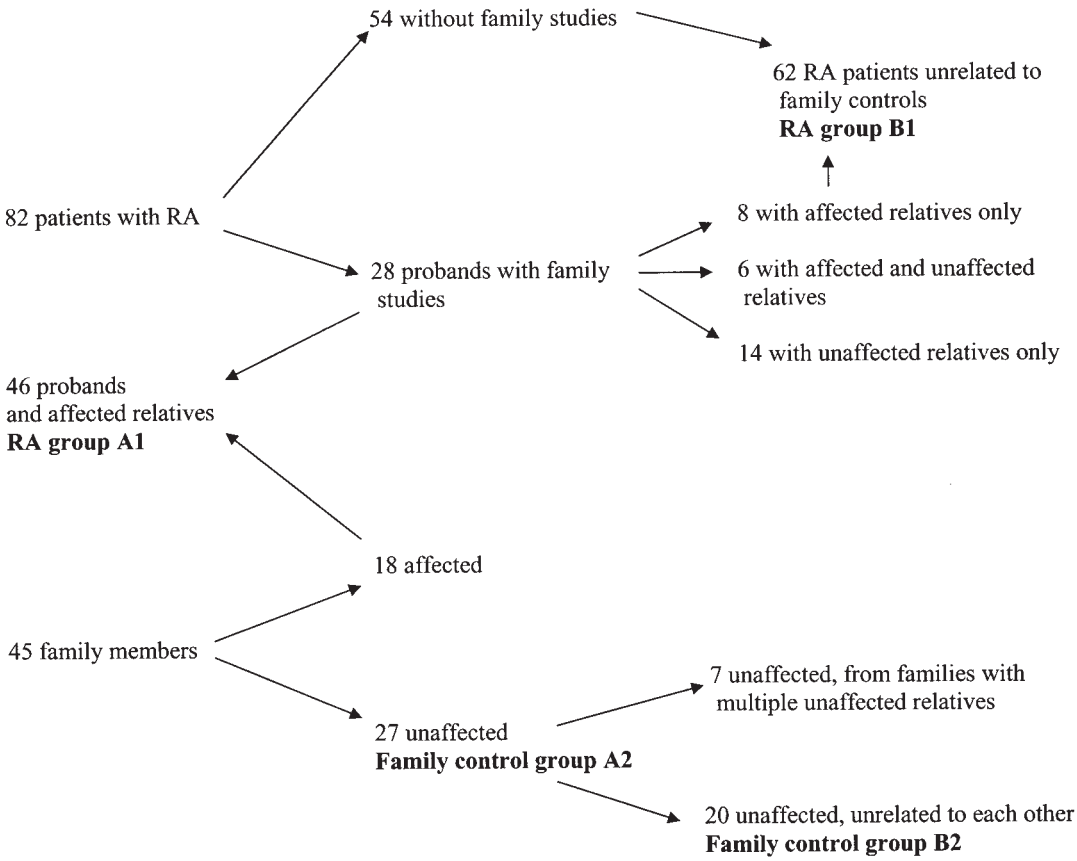

Figure 1. The composition of subjects in each group. The name of each group is printed in bold type. The A groups consist of affected (RA group A1) and unaffected (family control group A2) family members in the 28 families. Subjects within and between the B groups are unrelated to each other.

graphic characteristics of all affected subjects are shown in Table 1 and Figure 2. As shown, the median age at onset was 32 years; the median disease duration was 13 years. Almost all were ANA-positive, and most had erosive disease. The requirement for arthroplasty was high (21%) and the degree of self-reported disability on the HAQ was also high (median HAQ score 1.0).

Fourteen patients had disease onset at age < 16 years. Among these the median age at onset was 11.4 years. Patients with juvenile onset were younger at the time of study and had a trend toward lower frequencies of positive ANA (86% vs 99%; p = 0.051) and nodules (15% vs 44%; p = 0.070); but no differences were detected in disease duration, HAQ scores, number of DMARD used (Table 1), joint damage on radiographs (77% vs 84% for erosions and 100% vs 81% for joint space narrowing), frequency of arthroplasty (21% vs 22%), or sex distribution (86% vs 77% female) in comparison with patients with adult onset disease.

Multiplex families. Among 28 families studied, there were 18 affected and 27 unaffected relatives. At the time of study, unaffected relatives had a median age of 45 years (Table 2). Multiple members were affected in 14 families (Table 2). Therefore the familial prevalence of RA among the 28 studied families was 50% (14/28; 95% CI 30%, 70%). An additional 13 of the 28 probands had affected relatives by history. These relatives were not studied. If the total cohort of 82 patients are considered, the minimal familial prevalence of RA would be 0.17 (14/82; 95% CI 0.09, 0.25).

All but 2 affected relatives were first-degree relatives of probands, and sisters were the most commonly affected. Ten families had 2, and 4 families had 3 affected members (Table 2). Affected members were in the same generation in 8 families, and in 2 successive generations in 6 families (Table 2). Among the latter, the age at onset was significantly younger for those in the second generation; however, when only the 4 parent-child pairs were compared, the difference was only suggestive (p = 0.055; Table 2).

Shared epitope. Within the 28 families studied, the SE was found in 90.9% of probands and affected relatives (RA group A, Figure 1) and in 92.6% of unaffected relatives (family control group A2, Figure 1). There were no differences in the number of SE alleles between affected and unaffected members (percentages of individuals with 0, 1, and 2 SE alleles in each group were 9%, 61%, and 30% for

Table 1. Patients and relatives with RA or seropositive polyarticular JIA.

	All Subjects with RA or RF+ Polyarticular JIA	RF+ Polyarticular JIA	RA	RA vs RF+ Polyarticular JIA, p
N	100	14	86	
Female:male	78:22	12:2	66:20	NS
Age at onset, median (range), yrs	32.3 (6.8–63.1)	11.4 (6.8–15.1)	34.7 (16.0–63.1)	< 0.0001
Current age, median (range), yrs	48.0 (13.1–74.3)	28.1 (13.1–56.2)	49.4 (19.9–74.3)	< 0.0001
Disease duration, median (range), yrs	13.3 (2.2–41.4)	17.3 (2.2–41.4)	12.3 (2.3–40.0)	NS
HAQ score, median (range)	1.0 (0–3.0)	0.69 (0–2.0)	1.06 (0–3.0)	NS
No. swollen joints at time of study	4 (0–24)	5 (0–24)	4 (0–22)	NS
No. tender joints	5 (0–43)	5 (0–38)	4.5 (0–43)	NS
No. DMARD used, median (range)	3 (0–8)	2.5 (1–5)	3 (0–8)	NS
No. (%) with SE	87 (89.7)	12 (85.7)	75 (90.4)	NS
No. (%) with				
0	10 (10.3)	2 (14.3)	8 (9.6)	NS
1	51 (52.6)	8 (57.1)	43 (51.8)	
2 SE alleles	36 (37.1)	4 (28.6)	32 (38.6)	

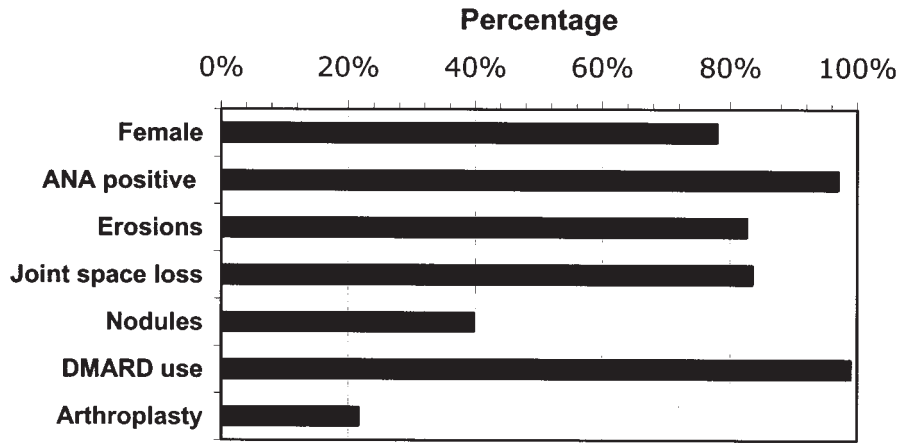

Figure 2. Frequencies of each characteristic in all 82 patients and 18 affected relatives. ANA: antinuclear antibody, DMARD: disease modifying antirheumatic drugs.

RA group A1; and 7%, 59%, and 33% for family control group A2; p = not significant). The number of SE alleles shared between probands and affected relatives or between probands and unaffected relatives was also not different. Of the 16 affected relatives, 3 (19%) shared none, 12 (75%) shared one, and one (6%) shared 2 SE-positive alleles with probands; while among 27 unaffected relatives, 8 (30%) shared none, 15 (56%) shared one, and 4 (15%) shared 2 SE-positive alleles with probands (p = not significant).

Cytokine genotypes. The frequency of homozygous and heterozygous genotypes for each cytokine allele among the groups analyzed below are shown in Table 3. The only differences were found in the distribution of IL-10 promoter –1082 genotypes between all unaffected relatives (family control group A2, Figure 1), and probands and affected family members (RA group A1, Figure 1; uncorrected p = 0.027), and between unaffected relatives (family control group B2, Figure 1) and unrelated patients (RA group B1, Figure 1; uncorrected p = 0.003) (Table 3).

Cytokine genotypes and SE. To determine whether any cytokine genotypes may affect the risk of RA when controlling for the SE, a multiple logistic regression analysis was performed. Within the families studied, only the heterozygous IL-10 –1082 G/A genotype decreased the odds of RA relative to the A/A genotype (OR 0.247 or a 75% risk reduction, 95% CI 0.081, 0.751; p = 0.014; Table 4). The SE and other cytokine polymorphisms tested were eliminated in the regression (Table 4). When a conditional logit analysis,

Table 2. Families studied.

	Affected Probands and Relatives	Unaffected Relatives
No. of probands	28	NA
No. of relatives	18	27
F:M	40:6	22:5
No. of families with		
2 members affected	10	
3 members affected	4	
Relation of relatives to probands		
Sister	11	14
Brother	1	3
Daughter	3	2
Son	0	1
Father	1	1
Mother	0	6
Niece	2	0
No. of families		
with 1 generation affected	8	
with 2 generations affected	6	
Age at onset in multiplex families with 2 generations affected median (range), yrs		NA
1st generation, n = 8	32 (17–62)*	
2nd generation, n = 6	21 (12–40)**	
Parent-child pairs, n = 4		
Parent	39 (17–52)***	
Child	22 (12–40)	
Age at study, median (range), yrs	47.5 (13.1–74.3)	45.1 (15.4–59.5)

* Paired t test comparing affected members in 1st and 2nd generations, for 8 pairs using 2nd generation member more than once in same family, p = 0.007; ** using 6 pairs of relatives, p = 0.017; *** comparing parent and child pairs, p = 0.055. NA: not applicable.

making comparisons within each family, was done, the power was reduced, and the G/A genotype showed only a trend toward a reduction in risk. Results for this calculation were as follows: for the –1082 G/A genotype, the OR was 0.316 (95% CI 0.087, 1.151; p = 0.0807) and for the G/G genotype, 0.349 (95% CI 0.048, 2.544; p = 0.2999).

Since relatives may be haploidentical, the results were confirmed by analysis of unrelated subjects to eliminate this bias. Patients whose families were not studied and those who had no unaffected relatives in the study were entered as the affected group (RA group B1, Figure 1) and unaffected relatives of other probands were the control group (family control group B2; Figure 1, Table 4). In these analyses the IL-10 –1082 G/A genotype gave an OR of 0.203 or an 80% risk reduction (95% CI 0.064, 0.640; p = 0.006) and the G/G genotype yielded an OR of 0.093, a 91% risk reduction (95% CI 0.013, 0.676; p = 0.019; Table 4). Again, the SE and other cytokine genotypes were eliminated.

DISCUSSION

RA is believed to have originated in the indigenous North American population and still occurs at high frequencies in present-day NAN populations[20,21]. High frequencies of both RA and the SE have been reported in several tribal groups widely scattered in North America. These include the Chippewa in Minnesota, Pima in Arizona, Tlingit in Alaska, Yakima in Washington State, and the present Cree and Ojibway Algonkian tribes from the prairie region in Manitoba and the neighboring areas in Northwestern Ontario[20,22-27]. The specific SE-bearing alleles differ in these groups. For example HLA-DRB1*1402 predominates in the Tlingit, Yakima, and Pima, whereas DRB1*04 alleles predominate in the Chippewa and both DRB1*1402 and DRB1*04 alleles are found in the Cree and Ojibway[20,24,30,49-52].

The patients studied were genetically a relatively homogenous group, the majority having 4 NAN grandparents. A high concentration of RA-predisposing genes in this cohort was suggested by the relatively young age at onset and severe disease phenotype, e.g., the majority of cases had erosive, destructive disease, a high proportion required arthroplasty, and HAQ scores were high. The familial prevalence of RA was 50% among the 28 studied families, based on relatives whose diagnosis was confirmed in this study. An additional 13 probands had a positive family history of RA and therefore the familial prevalence is potentially as high as 96% (27 of 28). There was a potential bias in the selection of relatives, as those who were studied were more easily accessible.

There were few affected parent-offspring pairs and there was only a trend toward a younger age at onset in offspring compared to affected parents. However, this observation is consistent with other reports suggesting genetic anticipation in RA, but the mechanism underlying this phenomenon in RA is unclear[53,54]. Although patients with polyarticular RF-positive JIA were included with the RA group in the analyses, it is possible that distinct genetic predispositions may account for a juvenile onset.

Although unaffected relatives may still develop RA, their median age at study was more than a decade higher than the median age at onset of disease for patients and affected relatives. As may be expected, the frequency of the SE was higher among unaffected relatives than the 59% frequency previously reported for unselected controls from this population[30]. The use of family controls allows better ethnic matching with cases, minimizing the effect of population admixture. In this setting, the SE did not affect the risk of RA within affected families.

The only significant protective element discovered was genotypes with the IL-10 promoter –1082 G allele. A protective effect of the G/A genotype was discovered by analysis within affected families, and analysis of unrelated cases and controls confirmed that both G/A and G/G genotypes reduced the risk of RA. However, in each case the control group were unaffected relatives of probands. Additional associations may have been missed due to inadequate power

Table 3. Frequencies of cytokine genotypes and allele frequencies.

	Probands and Affected Relatives in Studied Families (RA Group A1)	Unaffected Relatives (Family Control Group A2)	Unrelated Patients (RA Group B1)	Unaffected Relatives (Family Control Group B2)
No. studied	43	27	62	20
TNF-α -308 G→A, % with genotype				
G/G	83.7	92.6	87.1	95
G/A	14.0	7.4	11.3	5
A/A	2.3	0	1.6	0
Allele frequencies				
G	0.093	0.037	0.073	0.025
A	0.907	0.963	0.927	0.975
TFG-ß1 codon 10, % with genotype				
T→C				
C/C	18.6	11.1	14.5	10.0
C/T	46.5	48.1	49.4	50.0
T/T	34.9	40.7	37.1	40.0
Allele frequencies				
C	0.581	0.648	0.613	0.650
T	0.419	0.352	0.387	0.350
TGF-ß1 codon 25, % with genotype				
G→C	0	0	0	0
C/C	2.3	0	3.2	0
C/G	97.7	100	96.8	100
G/G				
Allele frequencies				
C	0.988	1.0	0.984	1.0
G	0.12	0	0.016	0
IL-10 -1082 G→A, % with genotype				
A/A	62.8	37.0*	71.0	30.0**
A/G	20.9	51.9	25.8	55.0
G/G	16.3	11.1	3.2	15.0
Allele frequencies				
A	0.267	0.370	0.161	0.425***
G	0.733	0.630	0.839	0.575
IL10 -819 C→T, % with genotype				
T/T	25.6	25.9	29.0	20.0
T/C	48.8	48.1	54.8	50.0
C/C	25.6	25.9	16.1	30.0
Allele frequencies				
T	0.500	0.500	0.435	0.550
C	0.500	0.500	0.565	0.450
IL-10 -592 C→A, % with genotype				
A/A	25.6	25.9	30.6	20.0
A/C	48.8	48.1	53.2	50.0
C/C	25.6	25.9	16.1	30.0
Allele fequencies				
A	0.500	0.500	0.427	0.550
C	0.500	0.500	0.573	0.450
IL-6 -174 G→C, % with genotype				
C/C	0	0	0	0
C/G	18.6	14.8	14.5	20.0
G/G	81.4	85.2	85.5	80.0
Allele fequencies				
C	0.907	0.926	0.927	0.900
G	0.093	0.074	0.073	0.100
IFN-δ +874 T→A, % with genotype				
A/A	69.9	63.0	69.4	55.0
T/A	25.6	29.6	25.8	35.0
T/T	4.7	7.4	4.8	10.0
Allele frequencies				
A	0.174	0.222	0.177	0.275
T	0.826	0.778	0.823	0.725

Comparisons between groups were by chi-square of Fisher exact tests. Cytokine genotyping was missing for one proband and 2 affected relatives. * p = 0.027 for comparison of RA group A1 with unaffected relatives (family control group A2); ** p = 0.003 and *** p = 0.014 for comparisons of RA group B1 with family control group B2. See Figure 1 for identification of subject groups.

Table 4. Multiple logistic regression analyses.

Cox and Snell R^2 for Regression	Explanatory Variable	Odds Ratio	95% CI	p
RA group A1[a] versus family control group A2[b] in studied families				
0.094	IL-10 -1082 G/A	0.247	0.081, 0.751	0.014
	IL-10 -1082 G/G	0.897	0.193, 4.172	0.890
	Constant	2.600		0.010
RA group B1[c] versus family control group B2[d]				
0.126	IL-10 -1082 G/A	0.203	0.064, 0.640	0.006
	IL-10 -1082 G/G	0.093	0.013, 0.676	0.019
	Constant	7.167		< 0.0001

Variables entered into the logistic regression were: IFN-γ promoter 874, IL-10 promoter –592, IL-10 promoter –819, IL-10 promoter –1082, TNF-α promoter –308, TGF-ß1 condon 10, TGF-ß1 codon 25, IL-6 promoter –174 genotypes, and SE. For each cytokine polymorphism, homozygous high, heterozygous, and homozygous low-producing genotypes were entered, with the homozygous low-producing genotype as reference. Results shown are for forward conditional entry of variables. Backward entry methods produced identical results. See Figure 1 for identification of subject groups. [a] number (n) in these analyses = 42; [b] n = 27; [c] n = 61; [d] n = 20.

as the number of families studied was small. Further studies are also required to determine whether the observed associations occur within this population as a whole.

IL-10 is a unique protein that has antiinflammatory effects, modulating Th1 responses, and inhibiting macrophage production of IL-12 and TNF and expression of MHC molecules[35]. However, it also stimulates humoral immunity and thus may promote autoimmunity[35,44]. The concept that cytokine production may be correlated with gene polymorphisms is attractive, as it suggests the possibility of genetic control of immune responses. Although IL-10 –1082G –819C –592C (GCC) constructs have been associated with higher rates of transcription than other haplotype constructs, reports of associations of *in vitro* protein synthesis with cytokine genotypes in general, and for IL-10 genotypes in particular, have been controversial[40,41,45,55,56]. This controversy may relate to differences in methodology and the subjects studied[55]. For example, compared to other haplotypes the IL-10 –1082G –819C –592C haplotype has been associated with higher *in vitro* IL-10 synthesis in most studies, but there are exceptions[40,41,55,56]. Further, *in vivo* production at sites of disease, such as the synovium in RA, may be difficult to predict from *in vitro* correlations, due to local cellular interactions and cytokine milieu.

Regardless of these factors, various IL-10 polymorphisms have been associated with risk or severity of RA in other populations. Recent analysis of a genome-wide scan of families with RA in Europe revealed linkage of an IL-10 allele, but only among sibling pairs who were HLA identical or who had erosive disease[57]. Although the evidence was weak, these findings suggest possible interactions between HLA and IL-10 and may support a role of IL-10 in disease severity[57]. Other reported associations include an increased risk of RA with an IL10 microsatellite allele, R2[58], an association of IgA RF with the IL-10 –1082A allele[43], greater joint damage with IL-10 –2849G/G genotype[44], and less joint damage with IL-10 –1082A/A genotype[45].

Other cytokine genes that have been found to affect the risk or severity of RA in other populations include TGF-ß, IL-6, IL-4, and TNF-α polymorphisms[6-14]. However, some of these associations have been controversial[15-19]. The failure to find associations with other cytokine alleles in our study may be due primarily to the selection of our controls and inadequate power (see above). Additional reasons for the discrepancy are differences in the particular polymorphisms studied, ethnic differences in allele frequencies, and the effect of population stratification in other case-control studies. Similarly, ethnic differences may affect the generalizability of the particular findings of this study to other unselected populations.

In summary, the phenotype of RA in a clinic cohort of North American Native patients was one of early onset and severe destructive arthritis with poor functional outcome. There was a high familial incidence of RA, and in multicase families there was a trend toward younger age at onset in later generations. The frequency of the SE was exceedingly high in both affected patients and unaffected relatives; and within affected families, the occurrence of RA was influenced by IL-10 genotypes, rather than by the SE. Because of the uncertainty of functional correlations with IL-10 genotypes it is unclear whether the protective effect of IL-10 promoter –1082 G/A genotype is due to differentials in IL-10 production. Although our study was limited by the small number of families we investigated, the results show that additional genes that may affect the risk of RA can be identified within highly predisposed families.

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