Rheumatoid arthritis susceptibility and interleukin 10: a study of two ethnically diverse populations

K. MacKay, A. Milicic, D. Lee, M. Tikly¹, S. Laval, J. Shatford and P. Wordsworth

Introduction. IL-10 is an immunoregulatory cytokine which may modulate disease expression in rheumatoid arthritis (RA). The IL-10 gene is highly polymorphic with a number of single nucleotide polymorphisms in the promoter region and two microsatellite loci, IL10.R and IL10.G, 4 kb and 1.1 kb 5' of the transcription initiation site. It has been reported that allele 2 of the IL10.R microsatellite (IL10.R2) is associated with increased IL-10 secretion and IL10.R3 with reduced secretion. Subsequently, over-representation of IL10.R2 and under-representation of IL10.R3 in three independent RA groups has been reported. The aim of the current study is to determine whether there is an association between the IL10.R2 allele and RA in two ethnically distinct populations.

Methods. IL10.R genotypes were determined by semi-automated DNA sequencing technology in 186 UK Caucasians and 138 South Africans of Zulu or Sotho origin, fulfilling the 1987 American College of Rheumatology (ACR) criteria for RA. The Caucasian patients had relatively severe disease and comprised 75 patients with RA vasculitis, 22 with Felty's syndrome and 89 who had undergone a joint replacement (hip or knee) within 15 years of the onset of disease. Allele frequencies were compared with 296 Caucasians and or 73 South Africans.

Results. The frequency of the IL10.R2 allele was significantly greater in the South Africans (RA and controls) than in the Caucasians (0.78 vs 0.66, \( P=1 \times 10^{-6} \)), while the frequency of IL10.R3 was less common (0.16 vs 0.3, \( P=1 \times 10^{-8} \)). No differences were observed in either IL10.R2 or IL10.R3 frequencies between patients and controls in either population.

Conclusions. We were unable to confirm any association between IL10.R alleles and RA in this study. However, significant differences were demonstrated in the frequency of IL10.R2 and IL10.R3 between the two ethnic groups. The relatively high frequency of IL10.R2 in the South African population (0.78) would have reduced the power to detect an association with RA.

Key words: Rheumatoid arthritis, Interleukin 10, Disease susceptibility, Ethnic diversity.
production in RA [3]. Consequently, the potential beneficial effects of anti-inflammatory cytokines such as interleukin 10 (IL-10) [4] and interleukin 4 (IL-4) [5] in RA are of great interest.

The immunomodulatory cytokine IL-10 is produced by a variety of cell types, including monocytes [6] and B lymphocytes [7]. It is a potent up-regulator of B-cell production and differentiation [8], but has anti-inflammatory capabilities that can directly down-regulate TNFz, IL-1, IL-8 and interferon-γ production [6, 9]. Variation in IL-10 secretion is largely genetically determined [10] and differences in secretion in RA have been associated with specific chronic inflammatory and infectious diseases. High levels of secretion are associated with a poor or fatal outcome in meningitis [10] and low levels are associated with susceptibility to severe malarial anaemia [11].

The IL-10 gene maps to the junction of 1q31–q32 [12] and exhibits substantial polymorphism in the promoter region which appears to correlate with variation in transcription [13, 14]. Two microsatellite polymorphisms, IL10.G and IL10.R, situated 1.1 and 4 kb 5’ of the transcription initiation site, respectively, are of particular interest [15, 16] since variation in IL-10 secretion has been associated with particular haplotypes defined by these microsatellite markers. Haplotypes containing the IL10.R2 allele are associated with higher levels of secretion than those including IL10.R3 [17]. Eight single nucleotide polymorphisms (SNPs) have also been identified in the promoter region of this gene [18–20], three of which have been studied in some detail: −1082 (G to A), −819 (C to T) and −592 (C to A) [13, 18, 20]. Increased IL-10 secretion has been described with the common GCC haplotype and reduced IL-10 secretion with the least common ATA haplotype, but direct comparisons between these studies are difficult as they have employed different experimental protocols [13, 20].

Various IL10.R, IL10.G and SNP genotypes have been reported to show association with a variety of chronic inflammatory diseases, including the ATA haplotype with extended oligo-articular juvenile idiopathic arthritis [13], IL10.G with systemic lupus erythematosus [21] and recently the IL10.R2 allele with RA [22].

This study was undertaken to investigate further the association of IL10.R2 with RA in UK Caucasians and to determine if it extended to another racially distinct population.

Materials and methods

Patients and controls

Two groups of racially distinct patients with RA and ethnically matched controls were recruited from the United Kingdom and South Africa. The patients consisted of 186 Caucasian and 138 South African (SA) patients of Sotho or Zulu ethnicity, fulfilling the American College of Rheumatology (ACR) 1987 criteria for RA. The Sotho and Zulu groups have a similar genetic background [23]. All the UK Caucasian patients had relatively severe disease and comprised 75 patients with RA vasculitis, 22 with Felty’s syndrome and 89 who had undergone a large joint replacement (hip or knee) within 15 years of the onset of disease. The mean age (± s.d.) of the Caucasian RA patients was 64 (± 11.6) yr and 70 per cent were female. Mean disease duration was 18.7 (± 10.5) yr and mean age of disease onset was 44.9 (± 14.5) yr. The SA patients were not chosen specifically for disease severity but were all recruited from a tertiary hospital out-patient clinic and required disease-modifying therapy. The mean age (± s.d.) of the SA patients was 49.5 (± 10.9) yr, average disease duration was 7.6 (± 7.3) yr and 76 per cent were female. The UK Caucasian control group consisted of 210 healthy blood donors and 86 unaffected spouses of patients attending a skeletal dysplasia clinic. The South African controls included 73 healthy, ethnically matched hospital workers or hospital out-patients attending with minor trauma.

IL10.R genotyping

Genomic DNA was extracted from peripheral venous blood samples using standard techniques. The IL10.R microsatellite was amplified by polymerase chain reaction (PCR) (primers were 5’ CCC TCC AAA ATC TAT TTG CAT A (upstream) and 5’ CTC CGC CCA GTA AGT TTC ATC (downstream), the latter being tagged with a fluorescent dye (HEX)). Reactions were performed in 96-well plates (Costar) in 10 ml reactions consisting of 50 ng DNA, 400 nM each primer, 50 mM dNTP, 2.0 mM MgCl2 and 0.2 units DNA polymerase (Bioline, UK) in the manufacturer’s NH4 buffer. The cycling conditions were 94°C for 1 min, annealing 60°C for 1 min, extension 72°C for 1 min, for 32 cycles. PCR products were diluted with water and separated by electrophoresis using an ABI 373 semi-automated sequencer (Applied Biosystems, Warrington, UK) and 6% denaturing polyacrylamide gels over 3 h. Products were sized using the program GENESCANTM Version 2.1 (Applied Biosystems, Warrington, UK) and genotypes semi-automatically assigned using the program GENOTYPERTM Version 1.1 (Applied Biosystems, Warrington, UK). All genotypes were then verified manually. The program GAS (Version 2) (A. Young, unpublished) was used to convert the size data into discrete allele numbers.

HLA-DR typing

Sequence-specific PCR, using 35 primers, was used to differentiate between the different HLA-DR alleles and undertake DR4 and DR1 subtyping [24].

Statistical analysis

Allele and genotype frequencies were calculated by direct counting. Since a common source of error in genotype assignment is the over-calling of homozygotes, Hardy-Weinberg equilibrium was used to predict the likely frequencies of IL10.R2 and IL10.R3 homozygotes and these figures were compared with the observed frequencies. IL10.R allele frequency distribution was compared between the Caucasians and South Africans and then between patients and controls. Subgroup analysis included disease severity (RA vasculitis, Felty’s syndrome or an early large joint replacement), sex, and shared epitope status (homozygosity or heterozygosity). The significance of differences between groups was calculated from contingency tables by χ² analysis. Odds ratios with confidence intervals were calculated.

Results

The frequency of the IL10.R2 allele was significantly higher (P = 1 x 10⁻⁵) in the South African population
overall (0.78) than the UK Caucasians (0.66), while the frequency of IL10.R3 was correspondingly reduced (0.16 vs 0.30, \( P = 1 \times 10^{-8} \)). However, no differences were observed in IL10.R allele frequencies between patients and controls in either racial group (Table 1). Eighty-four per cent of the UK Caucasian patients and 58 per cent of the SA patients were positive for the shared epitope.

A variety of subgroups were defined from the UK Caucasian RA cohort to analyse any possible associations with IL10.R alleles. All allele frequencies were very similar whether the groups were divided by sex, age of onset, shared epitope status, extra-articular disease (RA vasculitis and Felty’s syndrome) or early large joint replacement. The observed frequencies of IL10.R2 and IL10.R3 homozygous genotypes compared well with the predicted frequency of homozygotes by Hardy–Weinberg equilibrium suggesting alleles were being appropriately assigned.

### Table 1. Frequencies of IL10.R alleles in the South African and Caucasian populations studied

| Comparison groups | IL10.R alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles |Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Alleles | Allelesi  

<table>
<thead>
<tr>
<th>Study</th>
<th>Patient group</th>
<th>IL10.R2 % RA</th>
<th>IL10.R2 % control</th>
<th>IL10.R3 % RA</th>
<th>IL10.R3 % control</th>
</tr>
</thead>
<tbody>
<tr>
<td>This study</td>
<td>Caucasians with severe RA RA (n = 186)</td>
<td>68</td>
<td>65</td>
<td>28</td>
<td>31</td>
</tr>
<tr>
<td>This study</td>
<td>Black South Africans (DSND) RA (n = 138)</td>
<td>78</td>
<td>77</td>
<td>17</td>
<td>15</td>
</tr>
<tr>
<td>Lancet 1998 (Eskdale et al)</td>
<td>Glasgow Caucasians (DSND) RA (n = 103)</td>
<td>69</td>
<td>56</td>
<td>29</td>
<td>40</td>
</tr>
<tr>
<td>Lancet 1998 (Eskdale et al)</td>
<td>African Americans (DSND) RA (n = 61)</td>
<td>87</td>
<td>72</td>
<td>11</td>
<td>24</td>
</tr>
</tbody>
</table>

DSND, disease severity not documented.
Discussion

No association between IL-10 alleles and RA was apparent in the current study, in contrast to previous reports of an increase in IL10.R2 and a reduction in IL10.R3 alleles [22]. There are several possible explanations for this. First, the previous reported association with the IL10.R2 allele may be spurious. Second, it may be relevant that the IL10.R2 allele frequency in UK Caucasian controls in our study is significantly higher (0.65 vs 0.59, \(P=0.03\)) than that reported by Eskdale et al. and the IL10.R3 frequency significantly lower (0.31 vs 0.38, \(P=0.02\)). No differences in allele frequencies of IL10.R2 or IL10.R3 were apparent when the UK Caucasian patient groups from the two studies were compared (Table 2). This may suggest that IL-10 contributes a weak genetic effect but the relatively high frequency of IL10.R2 in the general population makes the effect more difficult to detect reliably. Since the frequency of IL10.R2 is even higher in South African Sotho and Zulu populations, the power to detect association with RA would be further reduced. Previously reported estimates of relative risk for IL10.R in RA range between 1.5 and 2.5 [22]. We estimate that our study in UK Caucasians had 80 per cent power to exclude an association between IL-10 and RA with an odds ratio of \(\geq 1.8\). However, in the South African study the higher frequency of the IL10.R2 allele (SA control frequency 77 vs 65% in Caucasians) means that the power to detect an effect with an odds ratio of \(\geq 1.8\) was only 36 per cent. In contrast the study had 80 per cent power to exclude a putative genetic effect with an odds ratio of \(\geq 3\). Third, genetic heterogeneity may be operating. The UK patients in this study were specifically selected for having more severe forms of RA and were only included if they had undergone an early large joint replacement or fulfilled the criteria for rheumatoid vasculitis or Felty’s syndrome. This is in contrast to the previous report where the main recruitment criteria was RA fulfilling the 1987 ACR criteria [22]. It is therefore likely that the disease severity of the Caucasian patients included in the two studies was different and it is conceivable that the IL10.R2 allele is not as strongly associated with severe forms of RA as it is with milder variants.

Inter-ethnic differences in IL-10 allele frequencies were not unexpected as similar differences have been described previously for the TNF locus [25–27] although in the case of TNF, linkage disequilibrium within the MHC may contribute to this variation [25].

A number of studies in normal individuals have demonstrated associations between IL-10 secretion and microsatellite or SNP polymorphisms characterizing distinct IL-10 haplotypes [17, 20]. Other studies have suggested that some of these haplotypes are associated with inflammatory diseases [13, 21, 28], although there is no really convincing evidence that these polymorphisms are directly involved in influencing IL-10 production or disease susceptibility. However, they may be markers for other relevant mutations within the gene and it is conceivable that only certain IL10.R2 haplotypes include specific SNPs associated with increased IL-10 secretion. Equally, only certain IL10.R3 haplotypes may truly be under-represented and associated with reduced IL-10 secretion. Discrimination between the various IL10.R2 or IL10.R3 extended haplotypes is not possible in a case-control study. As current evidence regarding a possible association of IL-10 with RA is inconclusive it would be appropriate to conduct a within-family association study to define the effects of specific haplotypes.

In conclusion, this study did not confirm an association between IL10.R2 and RA. However, as the data are inconclusive, further large studies investigating IL-10 as a candidate gene are justified but will require large numbers to achieve adequate statistical power. Extended haplotyping of the IL-10 promoter region should help to define any disease-causing haplotypes and so improve the chances of identifying an association.

Acknowledgements

Kirsten MacKay was an Arthritis Research Campaign Clinical Research Fellow, Stephen Laval was funded by the Oliver Bird Fund of The Nuffield Foundation and Anita Milicic is sponsored by the Grenville Bequest. The financial support of the Arthritis Research Campaign and the Nuffield Foundation is greatly appreciated by the authors. We are grateful to Drs D. G. I. Scott, R. Sturrock, R. Madhok and P. Bacon for allowing us to study their patients with rheumatoid vasculitis.

References


