SHORT COMMUNICATION

LACK OF ASSOCIATION BETWEEN AN INTERLEUKIN-1 RECEPTOR ANTAGONIST GENE POLYMORPHISM AND SYSTEMIC LUPUS ERYTHEMATOSUS

VICTOR A. DANIS^{*§}, MICHELLE MILLINGTON[§], QIRONG HUANG[‡], VALENTINE HYLAND[§]
AND DAVID GRENNAN[‡]

[§]*Kolling Institute of Medical Research, Royal North Shore Hospital, University of Sydney, St. Leonards, NSW 2065, Australia.*

[‡]*Department of Rheumatology, Royal North Shore Hospital, University of Sydney, St. Leonards, NSW 2065, Australia*

SUMMARY

Non-MHC linked genes may contribute to genetic predisposition to the development of systemic lupus erythematosus. The possibility that cytokine genes may be involved was raised by the observation of increased frequency in expression of an uncommon allele of an interleukin-1 receptor antagonist gene polymorphism and SLE in a recent U.K. study. We have not been able to show any significant differences in expression of this allele in SLE patients as a whole or in any patient subgroups. Our results actually show a slight decrease in the expression of this allele in SLE patients compared with healthy controls and in SLE patients with malar rash compared with SLE patients without malar rash.

KEY WORDS Interleukin-1 receptor antagonist Polymorphism SLE

INTRODUCTION

Systemic lupus erythematosus (SLE) is an autoimmune disease of unknown aetiology but with a genetic predisposition which is only partly accounted for by genes within the HLA region (Grennan *et al.*, 1995). In this situation there are a number of candidate genes which might be partly responsible for this undefined genetic predisposition to SLE including TNF α (Jacob, 1992) and interleukin-1 (Schorlemmer *et al.*, 1993). Polymorphisms in human cytokine genes have recently been described and initial studies have suggested that one of these polymorphisms, an uncommon allele of a variable repeat polymorphism in intron 2 of the IL-1ra gene (Tarlow *et al.*, 1993), may be associated with a number of epithelial diseases such as psoriasis (Cork *et al.*, 1993), ulcerative colitis (Mansfield *et al.*, 1994) and SLE (Blakemore *et al.*, 1993). In the latter study of patients with SLE, the uncommon allele (allele 2) appeared to be associated with particular disease manifestations, namely discoid rash and photosensitivity. In our study of SLE patients we did not find any increased expression of allele 2 in SLE patients as a whole,

*Correspondence to: Dr. V. A. Danis, Kolling Institute of Medical Research, Royal North Shore Hospital, St. Leonards, NSW 2065, Australia.

Table 1. IL-1ra genotypes and allele frequencies in healthy and diseased populations.

	Genotype						Allele Frequencies (%)		
	1,1	1,2	2,2	1,3	1,4	2,3	1	2	3 and 4
Healthy Controls (n=57)	28	18	6	2	1	2	68	28	4
RA (n=34)	16	13	2	3	0	0	71	25	4
SLE (n=41)	23	16	1	0	0	1	76	23	1

nor was there any increase in the frequency of expression of this allele associated with any of the disease manifestations or HLA DR haplotype. Moreover, our studies of the functional significance of this polymorphism suggest that this allele (associated with increased production of IL-1ar protein) should protect individuals from the development of SLE disease.

MATERIALS AND METHODS

Patients

41 unrelated subjects with SLE by criteria of the American Rheumatism Association (Tan *et al.*, 1982) were studied. Record was taken of the following criteria: malar rash, discoid rash, photosensitivity, oral ulcers, arthritis, serositis, renal disorder, neurological disorder, hematological disorder, immunologic disorder, antinuclear antibody (SSA and SSB), phospholipid antibody, and HLA DR type. In addition, 34 patients with definite rheumatoid arthritis and 57 healthy Anglo-Saxon Caucasian controls were studied.

PCR analysis of IL-1ra gene polymorphism

Genomic DNA was extracted from 20ml of EDTA blood by proteinase K digestion, phenol-cholorform extraction and ethanol precipitation using an established protocol (Wyam and White, 1980). 100ng of DNA was subjected to PCR amplification for the 86bp tandem repeat polymorphism of the IL-1ra gene in a GeneAmp PCR System 9600 (Perkin Elmer) using the primers as described (Tarlow *et al.*, 1993). Primers were synthesised using a 391 DNA Synthesiser (PCR-Mate, Applied Biosystems). DNA was denatured at 96°C for 60 seconds followed by 30 cycles of the following: 94°C 30 seconds, 60°C 30 seconds, 70°C 60 seconds. The final extension was carried out at 70°C for 7 minutes. PCR products were separated by electrophoresis on 2% agarose gels and visualised by ethidium bromide staining. A molecular ladder ranging from 67bp to 500bp (pUHpall, Bresatec) was used to determine the size of the PCR fragments.

Statistical Analysis

The results are presented as genotypes and allele frequencies. Fisher's Exact test for statistical significance was performed on allelic frequencies.

Table 2. IL-1ra genotypes and allele frequencies in SLE sub-groups. Fishers exact 2x2 frequency probability compared with the normal control population is shown in brackets.

	Genotype						Allele Frequencies (%)		
	1,1	1,2	2,2	1,3	1,4	2,3	1	2	3 and 4
Healthy Controls (n=57)	28	18	6	2	1	2	68	28	4
SLE:									
Malar Rash +ve (n=22)	14	6	1	0	0	1	77 (0.16)	20 (0.22)	2
Malar Rash -ve (n=15)	5	10	0	0	0	0	67 (0.63)	33 (0.79)	0
Discoid Rash +ve (n=1)	0	1	0	0	0	0			
Discoid Rash -ve (n=36)	19	15	1	0	0	1	74 (0.24)	25 (0.39)	1
Photosensitivity +ve (n=24)	11	12	0	0	0	1	71 (0.41)	27 (0.53)	2
Photosensitivity -ve (n=13)	8	4	1	0	0	0	77 (0.24)	23 (0.4)	0
Renal Proteinuria +ve (n=7)	3	4	0	0	0	0	71 (0.51)	29 (0.65)	0
Renal Proteinuria -ve (n=30)	16	12	1	0	0	1	73 (0.27)	25 (0.4)	2
Renal Cellular +ve (n=3)	2	1	0	0	0	0			
Renal Cellular -ve (n=34)	17	15	1	0	0	1	72 (0.32)	26 (0.48)	2
SSA +ve (n=8)	2	6	0	0	0	0	62 (0.76)	38 (0.86)	0
SSA -ve (n=29)	17	10	1	0	0	1	76 (0.17)	22 (0.27)	2
SSB +ve (n=3)	0	3	0	0	0	0			
SSB -ve (n=34)	19	13	1	0	0	1	75 (0.19)	23 (0.31)	2
HLA DR +ve (n=10)	3	7	0	0	0	0	65 (0.73)	35 (0.85)	0
HLA DR -ve (n=23)	12	9	1	0	0	1	72 (0.80)	26 (0.48)	2

RESULTS AND DISCUSSION

The IL-1ra genotype and allele frequencies in SLE patients and controls are summarised in Table 1. The frequency of allele 2 in SLE patients (23%) was only marginally lower than that in RA patients (25%) or controls (28%). This is in contrast to the reported increased frequency (35.5%) of the expression of this allele in SLE patients relative to controls (22.9%) by Blakemore *et al.* (1993). In our study, the frequency of expression of allele 2 in the normal Caucasian population (28%) was higher than that reported (23–24%) in the studies from the U.K. (Cork *et al.*, 1993; Mansfield *et al.*, 1994; Blakemore *et al.*, 1993). Moreover, we did not find any significant differences in the frequency of allele 2 expression in subsets of SLE patients categorised by malar rash, discoid rash, photosensitivity, renal manifestations, SSA and SSB antibodies, or HLA DR3 expression (see Table 2). The frequency of expression of allele 2 was lower in SLE patients with malar rash compared with those without malar rash but the difference failed to reach statistical significance.

We have recently shown that expression of allele 2 in healthy controls is associated with increased production of IL-1ra protein by monocytes stimulated by GM-CSF *in vitro* (Danis *et al.*, 1995). From these data it would appear that increased expression of allele 2 would not be expected to be associated with inflammatory diseases nor with manifestations of disease that involve IL-1. Our results suggest that expression of allele 2 may be slightly decreased in SLE patients, which would be consistent with a protective role for this genotype in autoimmune diseases.

CONCLUSIONS

We find no evidence of associations between IL-1ra genotypes and SLE.

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