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VNTR Polymorphisms of the IL-1RN Gene: IL-1RN*1 Allele and the Susceptibility of SLE in the Malaysian Population

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ABSTRACT

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease resulting from a defect in immunoregulation. The occurrence of this disease is mainly due to genetic, environmental and endocrine factors. Due to the role of IL-1ra in the regulation of T-cell activation, we hypothesized that its encoding gene may be associated with the susceptibility to SLE in the Malaysian population. The polymorphisms for the IL-1RN gene were analyzed in 100 SLE patients and 100 matched normal healthy controls. In this study, it was noted that the IL-1RN*1 allele was significantly associated to the susceptibility of SLE (OR = 2.667, p = 0.019). The IL-1RN*2 allele in turn, showed an inverse association (OR = 0.313, p = 0.012). Furthermore, our preliminary study also revealed the distribution pattern of genetic polymorphisms for IL-1RN gene in the Malaysian population.

Keywords: gel electrophoresis, IL-1 receptor antagonist, PCR

INTRODUCTION

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease with extensive tissue damage resulting from antibody and complement-fixing immune complex deposition (Wallace and Hahn 1997). In this prototype multisystem disease, many parts of the body can be affected such as the kidneys, joints, skin, lungs, blood vessels, the heart and the nervous system. Thus, severe complications may present during the course of this disease, i.e., arthritis, myositis, vasculitis, pleuritis, pericarditis, kidney failure and occasionally neurological disorders (Wallace and Hahn 1997). In Malaysia, the estimated prevalence rate of SLE is 43 per 100,000 persons (Wang *et al*. 1997). This disease primarily affects more females than males with a sex ratio of about 10 to 20 women to men (Janeway *et al*. 2001). The predominance of this disease occurring among females can be explained by female hormones, particularly estrogen (Wallace and Hahn 1997). In addition to the gender difference, SLE also occurs more commonly among Hispanic and African-American women compared to their Caucasian counterparts (Gray-McGuire *et al*. 2000). The race and environmental conditions in this disease reflects SLE's multifactorial nature.

SLE is characterized by its polygenic nature, where up to 100 genes may be involved in its development (Sullivan 2000). This is supported by several reported cases, in which 4% are family-related (Fielder *et al*. 1983), while there is a concordance rate of 24 to 65% among monozygotic twin pairs studied (Sullivan 2000). The interleukin-1 receptor antagonist gene (IL-1RN) is mapped to the band q14-q21 in the human chromosome 2 (Patterson *et al*. 1993). It is characterized by its variable number of 86-bp tandem repeat (VNTR) polymorphism, located in the intron 2 (Tarlow *et al*. 1993). This gene encodes for a 22 to 25 kDa glycosylated interleukin-1 receptor antagonist protein (IL-1ra), which is a natural *in vivo* regulator for interleukin-1 (IL-1) (Dinarello and Thomson 1991). IL-1 is a cytokine secreted by activated macrophages and is responsible for the differentiation of naive T-cells into T-helper cells during T cell-mediated immune response (Manetti *et al*. 1994). In the normal inflammatory pathway, the pro-inflammatory action of the IL-1 is counter-balanced by the IL-1ra to prevent further uncontrollable inflammation (Dinarello 2000). There are two isoforms of IL-1ra, namely the secreted and intracellular isoforms (Arend *et al*. 1998). Both of these isoforms are responsible for maintaining the balance between IL-1 and IL-1ra (Arend 2002).

However, the normal balance between the IL-1 and IL-1ra has failed in the context of SLE patients (Dean *et al*. 2000). As a result, there is an unmanageable inflammation which leads to subsequent tissue damage, and this is the characteristic clinical manifestation of SLE. Owing to the possibility of IL-1ra contributing to the occurrence of SLE, the distribution pattern of VNTR polymorphism of the IL-1RN gene, and its association to the susceptibility of SLE are being investigated in the Malaysian cohort of SLE patients compared to normal healthy controls.

MATERIALS AND METHODS

Patient and control samples

Blood samples were collected from the University Malaya Medical Centre (UMMC) in Kuala Lumpur, Malaysia. A total of 100 SLE patients and 100 matched healthy control blood samples were collected with informed consent (Ethics Approval No: 380.1). These SLE patients had fulfilled the American College of Rheumatology (ACR) criteria in confirming the diagnosis of SLE (Tan *et al*. 1982). DNA was then extracted from all samples by using a conventional phenol-chloroform DNA extraction method (Sambrook and Russell 2001).

Polymerase chain reaction (PCR) and Agarose gel electrophoresis (AGE)

The PCR conditions and composition of each of the components in the PCR mixture were optimized and modified accordingly from a previous study, described by Cantagrel *et al*. (1999). The PCR conditions were as follows: denaturation step at 95°C for 30 sec,

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annealing step at 58°C for 30 sec and extension step at 72°C for 30 sec. This PCR cycle was repeated for a total of 35 cycles and the IL-1RN gene including the intron 2 region (consisting of the desired sequences) was amplified. After amplification, the PCR products were then separated and analyzed via 2% (w/v) AGE, with the 100 bp DNA ladder (Fermentas, USA) as the indicator for the size of DNA fragments produced. The resulting bands corresponded to the number of 86-bp tandem repeats were visualized using a UV transilluminator.

Statistical analysis

The statistical analysis involved the determination of allelic and genotypic frequencies, Chi-square (χ^2) test (2 × 2 contingency table), *p*, odds ratio (OR) and 95% confidence interval (CI) values (Dawson and Trapp 2004). The determination of the frequency of each allele and genotype revealed the distribution of the genetic polymorphisms in the Malaysian population. In the χ^2 test, the *p* value of less than 0.05 indicates a statistically significant association between the two compared parameters (Dawson and Trapp 2004). On the other hand, the OR values indicate the extent to which an allele or genotype contributes to the occurrence of a certain disease. The 95% CI shows the range where the OR values can be accepted (Dawson and Trapp 2004).

RESULTS

Post PCR, different lengths of amplified fragments will be produced, which correlates to the number of tandem repeats present in the gene. As described previously, there are five different alleles observed in the IL-1RN gene, i.e., IL-1RN*1 (4 repeats), IL-1RN*2 (2 repeats), IL-1RN*3 (5 repeats), IL-1RN*4 (3 repeats) and IL-1RN*5 (6 repeats) (Blakemore *et al*. 1994). These alleles will yield PCR products of 410, 240, 500, 325 and 595 bp, respectively (Cantagrel *et al*. 1999). According to the length of PCR products being observed in AGE, the type of allele(s) can then be determined. **Figs. 1** and **2** demonstrate the bands that were visualized on the 2% (w/v) agarose gel, which represents the types of alleles present in both the SLE and healthy control groups, respectively.

From the results obtained, IL-1RN*1 and IL-1RN*2 alleles were scored in both SLE and healthy control groups. However, only the SLE group reported the presence of the IL-1RN*3 allele, while the healthy control group samples showed an additional IL-1RN*4 allele.

Overall, the allelic and genotypic frequencies of IL-1RN*1 and IL-1RN*2 are shown in **Tables 1** and **2**, respectively.

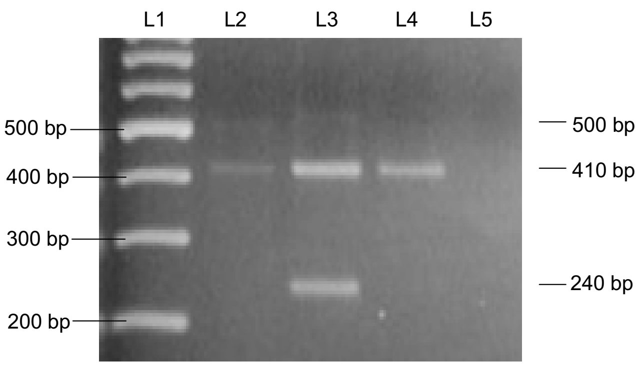

Fig. 1 EtBr-stained 2% (w/v) agarose gel of amplified IL-1RN gene polymorphisms for the SLE group. L1: 100 bp DNA ladder; L2: IL-1RN*1/IL-1RN*3 heterozygote; L3: IL-1RN*1/IL-1RN*2 heterozygote; L4: IL-1RN*1 homozygote; L5: negative control.

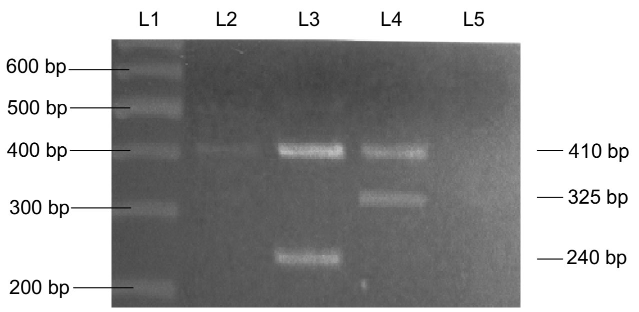

Fig. 2 EtBr-stained 2% (w/v) agarose gel of amplified IL-1RN gene polymorphisms for the healthy control group. L1: 100 bp DNA ladder; L2: IL-1RN*1 homozygote; L3: IL-1RN*1/IL-1RN*2 heterozygote; L4: IL-1RN*1/IL-1RN*4 heterozygote; L5: negative control.

DISCUSSION

The IL-1RN*1 is the most common allele in the Malaysian population, followed by the IL-1RN*2 allele. On the other hand, IL-1RN*3 and IL-1RN*4 are relatively rare. The IL-1RN*5 allele is not observed in either the SLE or healthy control groups. Thus, only four of the IL-1RN alleles are being observed in this population.

In this study, the frequency of the IL-1RN*1 allele revealed a significant association to SLE (χ^2 = 5.530, *p* = 0.019) (**Table 1**). Thus, we infer that there is a positive association between the IL-1RN*1 allele and SLE, on the assumption that the IL-1RN*1 allele may affect the production of both IL-1 and IL-1ra, with an increase in the production of IL-1 and/or a decrease in the IL-1ra production. In this context, there may be a relatively lower serum level of the IL-1ra compared to IL-1 and thus, there would then be insufficient IL-1ra to counter-balance the actions of IL-1. As expected, there may be some excessive action of IL-1, which could lead to the hyperactivation of T- and B-cells and subsequently, predispose an individual to SLE.

On the other hand, our results seem to differ with those obtained from previous studies, in which the IL-1RN*2 allele is instead found to be associated with SLE (Blakemore *et al*. 1994; Suzuki *et al*. 1997). In 1994, Blakemore and his colleagues demonstrated a novel association between the IL-1RN*2 allele and the presence of discoid rash and photosensitivity in SLE patients (Blakemore *et al*. 1994). Three years later, Suzuki and his research group demonstrated that IL-1RN*2 was linked to discoid skin rash, photosensitivity and the SLE Disease Activity Index (SLEDAI) score in SLE patients. SLEDAI is an indicator for the disease severity of SLE (Suzuki *et al*. 1997). It is used to measure the disease activity within the last 10 days in a SLE patient. The scoring of SLEDAI is done by referring to the specific manifestation in nine organ systems (Bombardier *et al*. 1992). Research conducted later reported a significant correlation between the IL-1ra and the musculoskeletal score of the British Isles Assessment Group (BILAG) disease activity index (Wais *et al*. 2003). The BILAG index is used to assess the clinical disease activity in SLE patients in the previous month. It is based on the physician intention to treat and contains the specific manifestations of eight organ systems (Symmons *et al*. 1988).

The frequency of IL-1RN*2 showed a significant increase (*p* = 0.012) in the healthy control group (**Table 1**). This indicates an inverse association between the IL-1RN*2 allele and SLE, and a possible protective effect of this allele to SLE. In this context, it is assumed that the IL-1RN*2 allele may affect the production of both IL-1 and IL-1ra, with a decrease in IL-1 production and/or an increase in IL-1ra production. Thus, there would be sufficient amounts of serum IL-1ra to counter-balance the physiological actions of IL-1, and eventually prevent the over-activity of IL-1. This phenomenon will help to maintain the normal intact immune response and subsequently, prevent the onset of SLE. Perhaps to a certain extent, the IL-1RN*2 allele may be protective against this disease. Parks *et al*. (2004) also reported that the IL-1RN*2 allele is not significantly associated to the risk of SLE. This seems to support our finding that the IL-1RN*2 allele is inversely associated to SLE susceptibility.

Table 1 The allelic frequency (n), χ^2 (*p*) and OR (95% CI) values of the IL-1RN gene polymorphisms in Malaysian SLE patients and normal healthy controls.

Allele	SLE patient n (%)	Normal control n (%)	χ^2 value (*p* value)	OR value (95% CI)
IL-1RN*1	192 (96)	180 (90)	5.530 (0.019#)	2.667 (1.146 – 6.207)
IL-1RN*2	6 (3)	18 (9)	6.383 (0.012#)	0.313 (0.121 – 0.805)
IL-1RN*3	2 (1)	0 (0)	2.010 (0.156)	-
IL-1RN*4	0 (0)	2 (1)	2.010 (0.156)	-
Total	200 (100)	200 (100)	-	-

$p^{\#}$ - statistically significant

Table 2 The genotypic frequency (n), χ^2 (*p*) and OR (95% CI) values of the IL-1RN gene polymorphisms in Malaysian SLE patients and normal healthy controls.

Genotype	SLE patient n (%)	Normal control n (%)	χ^2 value (*p* value)	OR value (95% CI)
IL-1RN*1/IL-1RN*1	92 (92)	80 (80)	5.980 (0.014#)	2.875 (1.201 – 6.883)
IL-1RN*1/IL-1RN*2	6 (6)	18 (18)	6.818 (0.009#)	0.291 (0.110 – 0.767)
IL-1RN*1/IL-1RN*3	2 (2)	0 (0)	2.020 (0.155)	-
IL-1RN*1/IL-1RN*4	0 (0)	2 (2)	2.020 (0.155)	-
Total	100 (100)	100 (100)	-	-

$p^{\#}$ - statistically significant

Table 3 Distribution of the IL-1RN gene polymorphisms in different ethnic populations. All data presented here are the frequency values in the percentage form.

Ethnic group	IL-1RN*1		IL-1RN*2		IL-1RN*3		IL-1RN*4		IL-1RN*5		Reference
	SLE	Control	SLE	Control	SLE	Control	SLE	Control	SLE	Control	
African-American	94.8	93.8	2.8	2.0	0.3	4.2	2.1	0.0	NIL	NIL	Parks *et al*. 2004
Caucasian (UK)	64.8	73.8	32.7	24.1	0.6	2.1	1.2	0.4	NIL	NIL	Blakemore *et al*. 1994
Caucasian (US)	82.0	85.4	16.3	11.9	1.7	2.7	0.0	0.0	NIL	NIL	Parks *et al*. 2004
Japanese	88.3	95.0	9.7	4.1	1.0	0.0	1.0	0.9	NIL	NIL	Suzuki *et al*. 1997
Malaysian	96.0	90.0	3.0	9.0	1.0	0.0	0.0	1.0	NIL	NIL	This study

Previously, there were a few studies which had demonstrated the association between the IL-1RN gene and SLE in various populations with different ethnic backgrounds (**Table 3**) (Blakemore *et al*. 1994; Suzuki *et al*. 1997; Parks *et al*. 2004). The distribution pattern of the IL-1RN gene polymorphisms in all of these populations is invariably similar to each other. Strangely, none of the populations studied demonstrated the presence of the IL-1RN*5 allele. Through comparison, it was found that the Japanese and Malaysian populations are parallel to each other in the distribution of polymorphisms pattern obtained (**Table 3**). This may be explained by a similar genetic background, i.e., possessing an Asian lineage, shared by both populations. On the other hand, comparable data was also obtained for both Caucasian populations of England and the US (**Table 3**) (Blakemore *et al*. 1994; Parks *et al*. 2004). As for the African-American population, a distinctive IL-1RN allelic distribution was obtained. These observations indicated the role of geographical and genetic factors in influencing the IL-1RN genetic polymorphisms distribution pattern. In addition, the association between a certain allele and the risk of disease may be different for diverse populations. As a result, there is a difference in the prevalence and susceptibility of SLE among different populations worldwide.

In fact, most previous studies carried out aimed to examine the association between the IL-1RN*2 allele with the clinical manifestation of SLE, due to the role played by IL-1 in mediating an inflammatory response (Blakemore *et al*. 1994; Suzuki *et al*. 1997). As a result, further studies should be carried out to demonstrate the association between the IL-1RN gene and disease expression with regards to the Malaysian SLE patients. In addition, the presence of a susceptible gene alone is insufficient to elucidate the occurrence of a disease. Thus, further research could be carried out at the mRNA and proteomic levels to demonstrate the influence of gene expression on disease susceptibility, severity and prognosis. For example, Sturfelt and his workgroup demonstrated the relationship between low levels of IL-1ra and renal involvement in the SLE patients during the disease course (Sturfelt *et al*. 1997). A similar study could then be carried out in the local population to further elucidate the association between IL-1ra and SLE at a different molecular level. Owing to the polygenic nature of this disease, it is suggested to further study other genes that are located in close proximity to the IL-1RN gene, i.e., the IL-1 alpha and IL-1 beta genes, with regards to the Malaysian population. Previously, Laurincova (2000) had demonstrated that the IL-1 alpha, IL-1 beta and IL-1RN genes are in linkage disequilibrium, where the expression of one gene might be regulated by the other two genes.

There is also an enormous interest on the potential of anticytokines in immunotherapy, owing to the vital role played by cytokines in autoimmune diseases. Broadly-speaking, anticytokines belong to a new family of biological response modifiers which regulate the *in vivo* function of cytokines. During disease, the balance between these cytokines and their natural anticytokines is upset and thus, lead to the occurrence of disease and pathogenesis. As a result, these anticytokines may serve as promising tools in future disease immunotherapy (Tartour *et al*. 1994). Since the US Food and Drug Administration had approved the use of IL-1ra for the treatment of rheumatoid arthritis in 2001 (Cohen 2004), it might then become an alternative treatment for SLE patients with lupus arthritis in the future, who are not responsive to conventional treatment (Ostendorf *et al*. 2005).

CONCLUSION

In this study, we found that IL-1RN*1 is the most common allele for the IL-1RN gene among the Malaysian population. In addition, the IL-1RN*1 allele is significantly associated to the susceptibility of SLE among Malaysians. The IL-1RN*2 allele, on the other hand, is inversely associated to SLE. The IL-1RN*2 allele also showed a significant association with the healthy control group samples, which might in turn, suggest its protective role in this disease with regards to the local population.

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