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,
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
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 allele and genotypic frequencies of IL-
1RN*1 and IL-1RN*2 are shown in Tables 1 and 2, respec-

DISCUSSION

The IL-1RN*1 allele 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 re-
vealed a significant association to SLE ($\chi^2 = 5.530$, $p = 0.019$) (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 produc-
tion of both IL-1 and IL-1ra, with an increase in the produc-
tion 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 bet-

The frequency of IL-1RN*2 showed a significant in-
crease ($p = 0.012$) 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 associ-
ated to the risk of SLE. This seems to support our finding
that the IL-1RN*2 allele is inversely associated to SLE sus-
cceptibility.

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 de-
sired sequences) was amplified. After amplification, the PCR pro-
ducts 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 corre-
sponded to the number of 86-bp tandem repeats were visualized
using a UV transilluminator.

Fig. 1 EthBr-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 EthBr-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.

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 pro-
ducts 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 con-
trol groups, respectively.

The statistical analysis involved the determination of allelic and
genotypic frequencies, Chi-square ($\chi^2$) 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 $\chi^2$
test, the $p$
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bation 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 cer-
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The IL-1RN*1 is the most common allele in the Malaysian
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that the IL-1RN*2 allele is inversely associated to SLE sus-
cceptibility.
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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**Table 1** The allelic frequency (n), \( \chi^2 \) (p) and OR (95% CI) values of the IL-1RN gene polymorphisms in Malaysian SLE patients and normal healthy controls.

<table>
<thead>
<tr>
<th>Allele</th>
<th>SLE patient (n, %)</th>
<th>Normal control (n, %)</th>
<th>( \chi^2 ) value (p value)</th>
<th>OR value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1RN*1</td>
<td>192 (96)</td>
<td>180 (90)</td>
<td>5.530 (0.019(^*))</td>
<td>2.667 (1.146 – 6.207)</td>
</tr>
<tr>
<td>IL-1RN*2</td>
<td>6 (3)</td>
<td>18 (9)</td>
<td>6.383 (0.012(^*))</td>
<td>0.313 (0.121 – 0.805)</td>
</tr>
<tr>
<td>IL-1RN*3</td>
<td>2 (1)</td>
<td>0 (0)</td>
<td>2.010 (0.156)</td>
<td>-</td>
</tr>
<tr>
<td>IL-1RN*4</td>
<td>0 (0)</td>
<td>2 (1)</td>
<td>2.010 (0.156)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>200 (100)</td>
<td>200 (100)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^*\) - statistically significant

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

<table>
<thead>
<tr>
<th>Genotype</th>
<th>SLE patient (n, %)</th>
<th>Normal control (n, %)</th>
<th>( \chi^2 ) value (p value)</th>
<th>OR value (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1RN<em>1/IL-1RN</em>1</td>
<td>92 (92)</td>
<td>80 (80)</td>
<td>5.980 (0.014(^*))</td>
<td>2.875 (1.201 – 6.883)</td>
</tr>
<tr>
<td>IL-1RN<em>1/IL-1RN</em>2</td>
<td>6 (6)</td>
<td>18 (18)</td>
<td>6.818 (0.009(^*))</td>
<td>0.291 (0.110 – 0.767)</td>
</tr>
<tr>
<td>IL-1RN<em>1/IL-1RN</em>3</td>
<td>2 (2)</td>
<td>0 (0)</td>
<td>2.020 (0.155)</td>
<td>-</td>
</tr>
<tr>
<td>IL-1RN<em>1/IL-1RN</em>4</td>
<td>0 (0)</td>
<td>2 (2)</td>
<td>2.020 (0.155)</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>100 (100)</td>
<td>100 (100)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^*\) - 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.

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>IL-1RN*1</th>
<th>IL-1RN*2</th>
<th>IL-1RN*3</th>
<th>IL-1RN*4</th>
<th>IL-1RN*5</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American</td>
<td>94.8</td>
<td>93.8</td>
<td>2.8</td>
<td>2.0</td>
<td>0.3</td>
</tr>
<tr>
<td>Caucasian (UK)</td>
<td>64.8</td>
<td>73.8</td>
<td>32.7</td>
<td>24.1</td>
<td>12.1</td>
</tr>
<tr>
<td>Caucasian (US)</td>
<td>82.0</td>
<td>85.4</td>
<td>16.3</td>
<td>11.9</td>
<td>1.7</td>
</tr>
<tr>
<td>Japanese</td>
<td>88.3</td>
<td>95.0</td>
<td>9.7</td>
<td>4.1</td>
<td>0.0</td>
</tr>
<tr>
<td>Malaysian</td>
<td>96.0</td>
<td>90.0</td>
<td>3.0</td>
<td>9.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Reference**

- Blakemore et al. 1994
- Suzuki et al. 1997
- Parks et al. 2004
ACKNOWLEDGEMENTS

This research work is supported by 36-02-03-6006 and FP047/2005C grants from the University of Malaya, Kuala Lumpur, Malaysia.

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