B-Cell Activating Factor Promoter Polymorphisms in Egyptian Patients with Systemic Lupus Erythematosus

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Abstract. Background: Systemic lupus erythematosus (SLE) is a heterogenous autoimmune disease involving most immune cells. Studies have revealed a number of cytokine pathways that play important roles in the disease process. Among these is B-cell activating factor (BAFF), which regulates B-cell maturation, survival, and function. Objective: To study the association between BAFF promoter polymorphism and systemic lupus erythematosus (SLE). Methods: Single nucleotide polymorphisms in the BAFF promoter region; -2841 (T>C), -2701 (T>A), -871 (C>T) were investigated by PCR-RFLP genotyping in fifty Egyptian SLE patients and thirty normal controls. Results: The frequency of mutant alleles of both -871C>T and -2701 T>A was higher among SLE patients than controls (p-value <0.001 and 0.000 respectively). There was a highly significant relationship between -871 C>T polymorphism and SLE (P<0.001), with the sensitivity and the specificity of the test being 100 %, and 70%, respectively. Patients expressing the -2701 T>A allele were seven times more prone to SLE than those with the T/T wild genotype (sensitivity of the test = 78%, specificity = 66.7%, odds ratio = 7.09, C.I at 95% = 2.29-22.64). Conclusion: Polymorphisms in the regulatory region of the BAFF gene do contribute to the susceptibility to SLE in Egyptian patients, which indicates BAFF as a potential therapeutic target.

Key words: SLE, BAFF, Polymorphism, 871 C>T, 2841 T>C, 2701 T>A.

Introduction

Various mechanisms have been suggested as drivers of systemic lupus erythematosus (SLE), including dysregulation of the immune system, overproduction of inflammatory cytokines, and impaired B- and T-cell tolerance and/or regulation [1-3]. B-cells have a central role in the pathogenesis of SLE; in addition to the well-known associations between SLE and specific autoantibodies, B-cells likely contribute to disease development via autoantibody-independent pathways [4].

In general, genetic predisposition is a significant factor in the development and expression of systemic autoimmune disease, and specific polymorphisms in B-cell signaling genes may be related to B-cell hyperactivity in SLE patients [5-7]. Genetic polymorphisms may be one of the driving forces behind the increased B-cell activating factor (BAFF) expression that is seen in patients with systemic autoimmune diseases [8,9].

BAFF belongs to the family of tumour necrosis factor (TNF) ligands and is expressed by various cell types, including monocytes, macrophages, neutrophils, dendritic cells, and T lymphocytes [10-12]. It binds to three receptors – BAFF receptor B-cell maturation antigen (BCMA), transmembrane activator and calcium modulating cyclophilin ligand [CAML] interactor (TACI), and BAFF-R/BLyS receptor 3 – which are primarily expressed on B lymphocytes [13,14]. The binding of BAFF with receptors induces immunoglobulin (Ig) class switching, cell proliferation, and increased survival of B cells [15]. In addition, excess amounts of BAFF result in the rescue of self-reactive B cells from anergy [16].

In SLE patients, s-BAFF levels are frequently elevated and associated with disease activity and elevated levels of autoreactive antibodies, including levels of anti-dsDNA antibodies [17-20]. However, the mechanisms responsible for increased s-BAFF levels in SLE are unclear [15].

An association has been reported between BAFF gene polymorphisms, changes in circulating BAFF protein levels, and changes in BAFF mRNA levels.
Polymorphisms in the BAFF 5’ regulatory region have been reported in patients with SLE and rheumatoid arthritis [21]. Moreover, polymorphisms in each of the 3 BAFF receptors (BCMA, TACI, and BAFF-R/BLyS receptor 3) have also been reported [24-28], which could affect the expression of BAFF at the protein and/or mRNA level [4].

Data on BAFF genotypes for non-Asian SLE patients are currently lacking, and the role of polymorphisms of the BAFF encoding gene has not been widely investigated in human SLE [29], possibly because this gene seems to be highly conserved [22,30]. However, there are data suggesting that the -871 C>T single nucleotide polymorphism in the 5’ promoter region of the BAFF gene increases disease susceptibility and circulating BAFF levels in patients with Sjögren’s syndrome [22,31].

The aim of the present work is to study the polymorphisms in the BAFF gene promoter (-871 C>T, -2841 T>C and -2701 T>A) in Egyptian SLE patients, as well as its significance in predicting the occurrence of SLE.

Methods

The study included 50 SLE patients (46 females and 4 males) attending the Rheumatology & Rehabilitation Department and Outpatients Clinic, Kasr Al Aini Hospital, Cairo University. Their ages ranged from 13-50 years. All patients fulfilled the revised and/or updated American College of Rheumatology criteria for the classification of SLE [32,33]. Thirty age- and sex-matched normal, healthy persons not suffering from any clinical diseases were included in the study as a control group. All participants gave written informed consent for the anonymous use of their data in compliance with the Helsinki Declaration.

Table 1. PCR RFLP genotyping of the Studied BAFF gene polymorphisms in SLE patients and control groups.

<table>
<thead>
<tr>
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<th>-871 C&gt;T polymorphism</th>
<th>-2841 T&gt;C polymorphism</th>
<th>-2701 T&gt;A polymorphism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients (N=50)</td>
<td>Controls (N=30)</td>
<td>Patients (N=50)</td>
<td>Controls (N=30)</td>
</tr>
<tr>
<td>Wild genotype</td>
<td>0(0%)</td>
<td>21(70%)</td>
<td>29(58%)</td>
</tr>
<tr>
<td>Homozygous gene</td>
<td>42(84%)</td>
<td>9(30%)</td>
<td>17(34%)</td>
</tr>
<tr>
<td>mutation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Heterozygous gene</td>
<td>8 (16%)</td>
<td>0(0%)</td>
<td>4(8%)</td>
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<tr>
<td>mutation</td>
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DNA extraction and analysis. Genomic DNA was extracted from peripheral blood samples by AXY Prep Blood Genomic DNA Miniprep Kit (Axygen, Biosciences, USA). Isolated DNA was stored at -20°C until used for PCR amplification.

PCR amplification. PCR amplification was performed in a final volume of 25 µl (5 µl DNA, 12.5 µl Taq PCR Green Master Mix, 1 µl each of primer, sense, and antisense, and 5.5 µl distilled water).

The PCR reaction was carried out in the DNA thermal cycler (PTC programmable thermal controller, MJ Research, Watertown, MA). The computerized thermal cycler was programmed for the following conditions:

-871C>T gene polymorphism: Initial denaturation cycle at 90°C for 5 minutes; 30 cycles of denaturation for 30 seconds at 95°C; annealing for 30 seconds at 57°C; extension for 4 minutes at 72°C; and final extension for 5 minutes at 72°C. The nucleotide sequence of the sense primer used was 5’ TTGTACACCGACCTGTTAGG 3’ and that of the antisense primer was 5’ TGGAAGTAAGTCCACTGGGAAT3’.

-2701T>A &-2841T>C gene polymorphisms: Initial denaturation cycle at 90°C for 1-3 minutes; 30 cycles of denaturation for 1 minute 95°C; annealing for 1 minute at 54°C; extension for 4 minutes at 72°C; and final extension for 10 minutes at 72°C. The nucleotide sequence of the primer used was as follows:

-2701T>A (sense primer): 5’ATTCCCTGTCAGAATTTTCTCT 3’ and (antisense primer): 5’CCTATAACTCCCACAATAAGGTGAC3’.

-2841T>C (sense primer): 5’ATTCCCTGTCAGAATTTTCTCT3’ and (antisense primer): 5’CCTATAACTCCCACAATAAGGTGAC3’.

Digestion of the amplified product by specific restriction enzyme for each polymorphism. 10 µl of the amplified product were mixed with 1 µl restriction enzyme (Ssi I enzyme [Fermentas, cod no #ER1791, lot no 00055787] for -871C>T and -2841T>C polymorphisms and Dpn II enzyme [Fermentas, code no # ER0811, lot no 00062809] for -2701T>A) and the mixture was incubated at 37°C for 16 hours.
The product was analyzed by gel electrophoresis using 2% agarose gel (Promega cat. No V3121) and ultraviolet light transillumination.

Results

The frequency of BAFF gene polymorphism was higher in the SLE patient group than in the control group, showing a statistically significant difference in -871C>T, and -2701T>A single nucleotide polymorphism (SNP) but a non-significant difference in -2841T>C SNP (p-value <0.001, 0.000, and >0.05 respectively).

As regards -871C>T SNP in SLE patients, none of the patients expressed the wild allele (homozygous CC) and 100% expressed the C>T mutation with the following frequency: 84% expressed the CT allele (heterozygous CT) and 16% expressed the TT allele (homozygous TT). While in the control group, 70% expressed the wild allele while 30% expressed the mutant allele (20% heterozygous TC and 10% homozygous CC). Comparing the frequency of expression of the wild and mutant alleles in both the patients and the control groups yielded p-values of 0.283, 0.180, and 0.759 respectively (Table 1 and Figure 1).

On the other hand, study of the -2841T>C SNP revealed no statistically significant difference between the patient and control groups. The frequency of expression in the patients was as follows; 58% expressed the wild allele (homozygous TT) and 42% expressed the mutant allele (heterozygous TC in 34% and homozygous CC in 8%). In the control group, 70% expressed the wild allele while 30% expressed the mutant allele (20% heterozygous TC and 10% homozygous CC). Comparing the frequency of expression of the wild and mutant alleles in both the patients and the control groups yielded p-values of 0.283, 0.180, and 0.759 respectively (Table 1 and Figure 3).

On the other hand, study of the -2841T>C SNP revealed no statistically significant difference between the patient and control groups. The frequency of expression in the patients was as follows; 58% expressed the wild allele (homozygous TT) and 42% expressed the mutant allele (heterozygous TC in 34% and homozygous CC in 8%). In the control group, 70% expressed the wild allele while 30% expressed the mutant allele (20% heterozygous TC and 10% homozygous CC). Comparing the frequency of expression of the wild and mutant alleles in both the patients and the control groups yielded p-values of 0.283, 0.180, and 0.759 respectively (Table 1 and Figure 3).

Discussion

Several previous studies have reported the association between BAFF gene polymorphism and elevated s-BAFF levels. BAFF promoter polymorphism lies in a putative binding site for nuclear factor (NF)-κB, which is known to enhance BAFF gene expression [22]. The NF-κB signaling pathway...
plays a pivotal role in regulating diverse aspects of immune function, including mediating inflammatory responses and facilitating adaptive immunity [34-36].

The BAFF -871T allele is associated with increased s-BAFF in Sjögren’s syndrome patients [31]. It was also reported (by Gottenberg et al.) that a significant association was observed between -871 C>T polymorphism and serum BAFF level: T allele carriers had a significantly higher BAFF level than did C allele carriers. Furthermore, BAFF protein levels were high in patients carrying two -871 T alleles, intermediate in patients with one T allele, and low in patients without a T allele [22]. Another study (by Emmerich et al.) reported that the -871 T/T genotype was found to be associated with very high levels of BAFF (median 1721 pg/ml), whereas the T/C and C/C genotypes were associated with normal levels of BAFF (median 703 pg/ml and 1037 pg/ml, respectively). In addition, the -871 T allele was associated with lower BAFF levels in controls [37]. These data suggest the presence of genetically modulated differences in BAFF regulation in health and autoimmune disease [31].

Given that previous reports have proved the association between BAFF gene polymorphism and elevated s-BAFF levels in autoimmune diseases other than SLE, and given that most of the current data on SLE are derived from animal models, we decided to study the frequency of different BAFF gene SNPs and their association with SLE in a group of Egyptian patients, with the intention of providing data on the association of BAFF gene polymorphism and susceptibility to SLE. Our study is the first to our knowledge that reports the presence of an association between BAFF gene polymorphism and SLE. In the study by Eilertsen et al., variations in the 5’ promoter region of the BAFF gene demonstrated no disease association with SLE in patients from Caucasian descent [29], which is in agreement with the only other study on this subject in a Japanese SLE cohort [21]. However, the discrepancy between previous results and our findings may be explained by variations in the SLE phenotype in different populations, which suggests that the findings in a certain population cannot be directly transferred to cohorts of different ethnicities.

A considerable body of evidence points to B cells playing a significant role in the pathogenesis of SLE [4]. Polymorphisms in the regulatory region of the BAFF gene do contribute to Egyptian patients’ susceptibility to SLE.

In mice, treatment with a BAFF antagonist was found to be beneficial in the context of either SLE or inflammatory arthritis [38-39]. In humans, both SLE and RA are associated with elevated circulating levels of BAFF [17,40], and s-BAFF levels in synovial fluids from affected joints are greater than those in the corresponding sera [41]. Previous studies of SLE documented significant correlations between BAFF expression and disease activity[42]. Additionally, a significant correlation was found between changes in circulating s-BAFF levels and changes in disease activity [19]. Patients with SLE have elevated serum levels of BAFF correlated with elevated levels of autoreactive antibodies [17].

In RA, B-cell depletion therapy is highly efficacious in a substantial percentage of patients despite the modest effects of such therapy on circulating auto-antibodies [43]. Given the crucial role of B cells in the pathogenesis of SLE and RA, it stands to reason
that factors which promote B-cell survival and/or function are also crucial [4]. A study by Morimoto et al., showed that the blockage of BAFF in T cell–B cell interaction reduced the production of autoantibody by TACI-Ig, which is a soluble decoy receptor for BAFF and APRIL [44]. Our findings and previous studies therefore suggest that, since polymorphisms in the regulatory region of the BAFF gene contribute to SLE susceptibility in Egyptian patients, BAFF antagonism may be an appropriate target for intervention.

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References


