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B-cell activating factor and related genetic variants in lupus related atherosclerosis

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ABSTRACT

Background: Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease with an increased atherosclerotic risk compared to healthy population, partially explained by traditional cardiovascular (CV) risk factors. Recent data suggest B-cell activating factor (BAFF) as an important contributor in the pathogenesis of both SLE and atherosclerosis. The aim of the current study is to explore whether serum BAFF levels along with variants of the BAFF gene increase lupus related atherosclerotic risk.

Patients-Methods: 250 SLE patients underwent assessment of plaque formation and/or intimal media thickness (IMT) measurements in carotid and femoral arteries by ultrasound. Disease related features and CV traditional risk factors were also assessed. Serum BAFF levels were determined by commercially available ELISA and five single nucleotide polymorphisms (SNPs) of the BAFF gene (rs1224141, rs12583006, rs9514828, rs1041569 and the rs9514827) were evaluated by PCR-based assays in all patients and 200 healthy controls (HC) of similar age and sex distribution. SLE patients were further divided in high and low BAFF groups on the basis of the upper quartile level of the distribution (1358 pg/ml). Genotype and haplotype frequencies in SLE patients and HC were determined by SNPStats and SHEsis software.

Results: High-BAFF SLE group displayed increased rates of both plaque formation and arterial wall thickening (defined as IMT > 0.90 mm) compared to patients with low BAFF levels (58.1% vs 43.6%, p:0.048 and 38.6% vs 23.2%, p-value: 0.024, respectively). The association remained significant after disease related features were taken into account (ORs [95%CI]: 2.2 [1.0–5.1] and 2.5 [1.1–5.5] for plaque formation and arterial wall thickening, respectively). Moreover, the presence of the AA genotype of the rs12583006 BAFF gene variant increased susceptibility for both lupus and lupus related plaque formation (ORs [95%CI]: 2.8 [1.1–7.1], and 4.4 [1.3–15.4] in the codominant model, respectively). Finally, the haplotype TTTAT was found to be protective for plaque formation among SLE patients (OR 0.3 [0.1–0.9]. No associations between BAFF gene variants with arterial wall thickening were detected.

Conclusions: High BAFF serum levels in the upper 4th quartile as well as BAFF genetic variants seem to increase susceptibility for both lupus and lupus related subclinical atherosclerosis implying B-cell hyperactivity as a potential contributor in the pronounced lupus related atherosclerotic risk.

1. Introduction

Systemic lupus erythematosus (SLE) is a chronic systemic autoimmune disease characterized by considerable heterogeneity in regard to the variety and severity of clinical and laboratory manifestations. Although genetic susceptibility along with environmental interactions contributes significantly to the immune dysregulation that characterizes SLE, the exact etiopathogenesis remains elusive. Epidemiological studies in SLE patients reveal a bimodal distribution in mortality rates, with the earlier peak attributed to infections and complications from kidney disease and/or neuropsychiatric lupus and a later peak mainly linked to atherosclerotic cardiovascular (CV) events

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[1]. A population-based case-control analysis, using general practice database data, found a 2-fold increase in CV disease risk for women with SLE [2], with a striking fifty-fold increased risk of myocardial infarction among SLE premenopausal women, not entirely explained by traditional risk factors for atherosclerosis [3]. These observations support the hypothesis that accelerated atherosclerosis and premature CV disease are significantly enhanced by factors inherent to the pathogenesis of SLE.

B-cell-activating factor (BAFF, also known as B lymphocyte stimulator-Blys) is a member of the tumor necrosis factor (TNF) superfamily, crucial for the survival, proliferation, differentiation, as well as antibody production [4]. SLE patients have increased serum BAFF levels in association with anti-DNA antibodies [5] and disease activity indices [6]. BAFF blockade by a monoclonal antibody-the first biological FDA approved agent for SLE-had considerable therapeutic results in alleviating several disease related features and reducing lupus activity [7,8]. Of note, a recent report in Sardinian population revealed a variant of the BAFF encoding gene (TNF superfamily member 13 b, TNFSF13B), as a novel risk factor for SLE. The latter has functional implications resulting in increased serum BAFF levels through generation of reduced binding sites of inhibitory microRNAs to BAFF mRNA [9].

BAFF and BAFF-receptor (BAFF-R) genetic variants have been previously studied in patients with autoimmune diseases including lupus [10,11] and Sjogren's syndrome [12–14]. In an earlier study by Kawasaki et al., the rs 9514828 BAFF polymorphism was found to be associated with the presence of anti-Sm as well as with BAFF mRNA monocyte levels in patients with lupus [10]. In subsequent studies by Nossent's group, distinct BAFF gene haplotypes were found to confer either increased risk for the primary SS subset characterized by the presence of anti-Ro and/or anti-La autoantibodies or elevated soluble BAFF levels [13], while such associations were not confirmed in lupus populations [11]. Taken together, these data imply genetic variations of BAFF as important contributors in lupus pathogenesis.

A growing body of data support a contributory role of B cells in pathogenesis of atherosclerosis in general populations and experimental models with conflicting results [15–19]. However, data on their effect in autoimmune populations is lacking. Given a possible role of BAFF in atherosclerosis, we sought to explore whether the serum levels of BAFF, along with the presence of previously described BAFF polymorphisms, are associated with markers of subclinical atherosclerosis-detected by intimal media thickness (IMT) and atherosclerotic plaque ultrasound evaluation- in SLE patients.

2. Patients and methods

Two hundred and fifty consecutive SLE patients followed up in the outpatient Rheumatology Clinics of two tertiary hospitals (“Laikon” General Hospital, ‘G.Gennimatas” General Hospital of Athens) in the area of Athens, Greece (mean age ± SD: 45.2 ± 13.2, female prevalence: 93.2%). All SLE patients fulfilled the revised American College of Rheumatology (ACR) 2012 criteria for the classification of SLE [20]. Two hundred age/sex matched healthy controls (HC) were also included (mean age ± SD: 48.1 ± 17.2, female prevalence: 94.0%). All patients and controls provided informed consent prior to the entry in the study and the study protocol was approved by the Ethics Committee of the National and Kapodistrian University of Athens and Laiko General Hospital of Athens. Patients with renal failure (defined as serum creatinine levels > 3 mg/dl, clearance creatinine < 30 ml/min), pregnant women, ages below 18 years old and previously treated with either belimumab or rituximab within the last year of enrollment, were excluded from the study.

3. Disease and traditional risk factors

Demographic data, disease activity/damage scores [(Systemic lupus erythematosus disease activity index (SLEDAI), Systemic lupus erythematosus international collaborating clinics (SLICC)], clinical and laboratory features as well as therapeutic regimens and traditional risk factors for atherosclerosis were recorded as previously described [21,22]. Storage of serum and whole blood was at −80 °C.

All 250 lupus patients underwent carotid and femoral arteries examination by ultrasound evaluation for detection of plaque formation (HDI 3500; Advanced Technologies Laboratories, Bothell, WA). In 225 patients who were willing to participate, IMT measurements were also performed as previously described [22]. Arterial wall thickening was considered present when IMT score was higher than 0.9 mm (IMT > 0.9 mm).

4. BAFF serum levels and BAFF genetic variants

Serum BAFF levels were measured using a polyclonal human BAFF enzyme linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol (Human BAFF/BLyS/TNFSF13B Quantikine ELISA Kit, R&D systems) in 250 SLE patients and 30 selected HC of similar age and sex distribution. Quality controls (low, medium and high) were purchased to ensure the reliability and accuracy of the BAFF measurements in each plate. All samples were diluted appropriately to run within the standard curve. Genomic DNA was extracted from blood samples using the Nucleospin Blood QuickPure kit (Macherey-Nagel GmbH & Co, Germany), according to the manufacturer's instructions. Five single nucleotide polymorphisms (SNPs) of the BAFF gene (rs1224141, rs12583006, rs9514828, rs1041569, the rs9514827) were evaluated by PCR-based assays, as previously described [14] in 250 SLE patients and 200 age sex matched HC.

5. Statistical analysis

Genotype and haplotype frequencies were determined in SLE patients and HC by SNPStats and SHEsis software [23]. Genotype frequencies in control subjects for each SNP were tested for departure from Hardy-Weinberg equilibrium [24]. Mann-Whitney and Fisher's exact tests were implemented for the comparison of numerical and categorical variables respectively between high and low BAFF level groups. Adjusted odds ratios (OR) and corresponding 95% confidence intervals (CI) were estimated by backward stepwise logistic regression taking into account disease variables emerged as potential confounders in univariate analysis (SPSS software). P-values of less than 0.05 and 0.1 were considered statistically significant for univariate and multivariate analysis respectively.

6. Results

6.1. Demographics and disease characteristics of study participants

Traditional risk factors for atherosclerosis and disease characteristics in lupus patients are displayed in Supplementary table 1.

6.2. BAFF levels in SLE patients and HC- association with markers of subclinical atherosclerosis in SLE

As shown in Fig. 1A, mean serum BAFF levels were found to be significantly higher in lupus patients vs HC (1248.0 ± 940.9 pg/ml vs 773.2 ± 142.5 pg/ml, $p < 0.0001$). We next sought to explore whether BAFF levels in the setting of lupus were associated with markers of subclinical atherosclerosis (plaque formation/arterial wall thickening). Mean serum BAFF levels in lupus patients with atherosclerotic plaque were found to be significantly higher compared to those without the plaque (1369.0 ± 1159 pg/ml vs 1140.0 ± 677.3 pg/ml, p-value: 0.029 Fig. 1B). Arterial wall thickening was also associated with higher BAFF levels, though the difference did not reach statistical significance (1460.0 ± 1398.0 pg/ml for patients with IMT > 0.9 mm vs