Kidney Res Clin Pract 35 (2016) 29–34

Kidney Research and Clinical Practice

journal homepage: http://www.krcp-ksn.com
Contents lists available at ScienceDirect

Original Article

Evaluating the association of interleukin-10 gene promoter -592 A/C polymorphism with lupus nephritis susceptibility

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Article history:
Received 31 May 2015
Received in revised form
15 October 2015
Accepted 10 November 2015
Available online 2 December 2015

Keywords:
Lupus nephritis
Promoter -592 A/C of interleukin-10 gene
Systemic lupus erythematosus

Abstract

Background: Interleukin-10 (IL-10) is an important immunoregulatory cytokine. There are few studies evaluating the association between IL-10 and lupus nephritis (LN). The aim of this study was to evaluate the association of IL-10 gene promoter -592 A/C with LN susceptibility.

Methods: The study was conducted on 84 patients with systemic lupus erythematosus (SLE). Patients were divided into LN group (Group I, 48 patients) and non-LN group (Group II, 36 patients). The -592 A/C polymorphisms in IL-10 promoter gene were determined by polymerase chain reaction and restriction fragment length polymorphism in both groups. IL-10 was determined by enzyme-linked immunosorbent assay. Frequencies of the genotypes were compared between LN and non-LN patients and among LN patients with different pathologic classes.

Results: There was a significant increase in serum level of IL-10 ($P = 0.001$) in Group I compared with Group II and significant positive correlation between serum IL-10 and SLE disease activity index ($r = 0.466$, $P = 0.001$) in Group I. There were no significant differences in the distribution of the IL-10 gene promoter -592 A/C genotypes or the allele frequencies between Groups I and II. There was no significant difference between AC/CC and AA genotypes with SLE disease activity index, proteinuria, hematuria, anti-double-stranded DNA, and IL-10 in Group I. There was no significant difference in the distribution of AC and CC genotypes among different pathologic LN classes.

Conclusion: IL-10 suggested to play a role in pathogenesis and development of LN. However, the promoter -592 A/C of IL-10 gene suggested to be not associated with serum IL-10 levels or LN susceptibility. In addition, it appears that promoter -592 A/C of IL-10 gene not associated with LN activity or the pathologic classes of LN.

Introduction

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease. Many factors are associated with the development of the SLE, including genetic, ethnic, immunoregulatory, hormonal, and environmental factors [1–4].

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http://dx.doi.org/10.1016/j.krcp.2015.11.002

The role of genetics in the development of SLE is supported by that SLE is more common in first-degree relatives of patients with SLE (familial prevalence, 10–12%). Prevalence rates are higher in monozygotic twins (24–58%) than in dizygotic twins (2–5%) [1–4].

The major and serious manifestation of SLE is lupus nephritis (LN). In most patients with SLE, LN is histologically evident, and kidney biopsy should be considered in any patient with SLE who has clinical or laboratory evidence of active nephritis, especially on the first episode of nephritis [5].

Many studies suggest that genetic predisposition plays an important role in the development of both SLE and LN. Multiple genes, many of which are not yet identified, mediate this genetic predisposition [1–4,6–9] .

Interleukin (IL)-10 is a cytokine with pleiotropic effects in immunoregulation and inflammation and produced mainly by monocytes and B lymphocytes [10,11]. IL-10 promotes B-cell-mediated functions, enhancing survival, proliferation, differentiation, and antibody production [12,13]. It also inhibits T cell function by suppressing the expression of proinflammatory cytokines such as tumor necrosis factor-α, IL-1, IL-6, IL-8, and IL-12 [14]. It also inhibits antigen presenting cells by down-regulating major histocompatibility complex Class II and B7 expression [14–17], which in SLE may contribute to impaired cell-mediated immunity.

In humans, the IL-10 gene is located on chromosome-1 and its receptor is located on chromosome 11 [18]. The IL-10 gene encodes for 5 exons. The IL-10 promoter is highly polymorphic, and in this region, 2 CA-repeat microsatellites (IL-10.G and IL-10.R) and 3 single nucleotide polymorphisms (SNPs), at positions −1082, −819, and −592 from the transcription start site, have been identified to correlate with IL-10 production [10]. Haplotypes comprising 3 SNPs at positions −1082, −819, and −592 have also been found to correlate with IL-10 serum level [10].

There are several studies suggesting that the IL-10 gene is associated with SLE susceptibility [19]. Studies in lupus animal models and humans have shown that anti-IL-10 treatment can decrease disease activity in terms of clinical features and biologic markers [20–22]. Interestingly, Llorente et al [23] demonstrated that IL-10 production by monocytes and B cells in healthy members of families with SLE was significantly higher than that of healthy unrelated controls, but was similar to that of SLE patients, thus suggesting that a genetically controlled high innate IL-10 production may predispose to SLE development [23]. Although there are several studies evaluating the association between IL-10 and SLE, the studies evaluating the association between IL-10 and LN are few.

Aim of the study

The aim of this study was to determine the distribution of the promoter -592 A/C of IL-10 gene in Egyptian patients with SLE and LN and evaluate the role of the promoter -592 A/C of IL-10 gene in the pathogenesis and clinical and histopathologic classes of LN.

Methods

This study was conducted on 84 patients with SLE who have the criteria of Systemic Lupus International Collaborating Clinics group [24]. Patients were divided into LN group (Group I, 48 patients with mean age 29.63 ± 8.91 years) and non-LN group (Group II, 36 patients with mean age 31.81 ± 0.20 years). The patients of both groups were matched for age, gender, and ethnic origin. LN was diagnosed clinically by the presence of persistent proteinuria or hematuria and confirmed by kidney biopsy. Non-LN patients were diagnosed according to the criteria of Systemic Lupus International Collaborating Clinics group [24], including arthritis, skin rash, positive antinuclear antibodies (ANAs), and positive anti-double-stranded DNA (dsDNA), but without renal involvement in the form of proteinuria, hematuria, or abnormal renal functions. SLE patients with proteinuria other than LN as pregnancy and fever or patients with impaired renal function due to any other cause than LN as diabetic nephropathy and patients with history of renal trasnsplantation or hepatitis C virus and hepatitis B virus and other connective tissue diseases other than SLE were excluded from the study. All these patients were selected from the nephrology outpatient clinics in nephrology departement, Theodor Bilharz Research Institute, Cairo, Egypt. The study was approved by the appropriate ethics committee and has therefore been performed in accordance with Declaration of Helsinki, and written informed consent was obtained from each patient participated in the study.

Each patient underwent thorough history taking and complete clinical examination.

Peripheral venous blood samples were collected from patients after proper disinfection.

(1) Two milliliters on EDTA for complete blood count.
(2) Five milliliters of blood in a plain glass tube left to clot at room temperature for 30 minutes then centrifuged for 10 minutes to obtain serum for chemical and immunological tests.

Routine examinations included urine analysis, renal function tests (serum creatinine, urea, sodium, potassium, and uric acid), complete blood count, erythrocyte sedimentation rate, and C-reactive protein. Serum C3 and C4, ANA, and anti-dsDNA were also conducted.

ANA was measured using indirect immunoflourescence. Anti-dsDNA were measured using solid-phase enzyme immunoassays kits. C3 and C4 were measured using Nephelometer (BN ProSpec, Dade Behring, Marburg, Germany).

Determination of -592 A/C polymorphisms in the IL-10 gene promoter

Genomic DNA was extracted from EDTA–whole blood using a phenol chloroform extraction method. The -592 A/C polymorphism in the IL-10 gene promotor was determined by polymerase chain reaction (PCR) and restriction fragment length polymorphism protocol using the following designed primer sequences:

5′ TCC AGC CAC AGA AGC TTA CAA C 3′ (forward);
5′ AGG TCT CTG GGC CTT AGT TTC C 3′ (reversed).

PCR was performed on a Gene-Amp PCR System 2700 (Applied Biosystems, Foster City, CA, USA) in the following conditions: 94°C for 5 minutes; 35 cycles of 94°C for 30 seconds, 64°C for 45 seconds, and 72°C for 60 seconds; followed by a final extending step at 72°C for 10 minutes. The PCR product