Cytokine 69 (2014) 272–276

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

Macrophage migration inhibitory factor (MIF) -173 polymorphism is associated with clinical erythema nodosum in Löfgren's syndrome

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ARTICLE INFO

Article history:
Received 19 September 2013
Received in revised form 7 April 2014
Accepted 21 May 2014
Available online 17 June 2014

Keywords:
Sarcoidosis
Löfgren's syndrome
Erythema nodosum
Macrophage migration inhibitory factor (MIF)
Single nucleotide polymorphism (SNP)

ABSTRACT

Introduction: Macrophage migration inhibitory factor (MIF) has been shown to be a key regulator in innate and adaptive immune responses. A single nucleotide polymorphism in the 5′ region of the MIF gene, *MIF* -173∗G/C, is associated with increased MIF protein production, in vivo and in vitro. Associations have been shown between the minor *MIF* -173C allele and sarcoidosis patients with erythema nodosum (EN). Löfgren's syndrome is an acute and usually self-remitting phenotype of sarcoidosis. It is defined as having an acute onset with bilateral hilar lymphadenopathy (BHL), fever, erythema nodosum (EN) and/or arthritis.

The aim of this study was to investigate whether *MIF* -173G/C associates with the susceptibility to and the clinical manifestations, i.e. arthritis or EN, of Löfgren's syndrome.

A total of 171 patients with Löfgren's syndrome and 313 controls were genotyped for a single nucleotide polymorphism at position -173 of the MIF gene (SNP rs755622), using a PCR and a restriction enzyme technique.

Results: There were no significant differences found in the *MIF* -173C allele frequencies between patients with Löfgren's syndrome and controls. In patients with Löfgren's syndrome with only EN, a significantly increased frequency of the C minor allele was observed compared to patients with arthritis only ($p = 0.0095$; OR 3.08, CI: 1.28–7.39).

Patients with only EN compared to patients with EN and arthritis showed a significantly increased frequency of the minor C allele ($p = 0.044$; OR 1.97, CI: 1.01–3.85). But patients with only arthritis compared to patients with EN and arthritis did not show a significant difference in C allele frequency ($p = 0.270$; OR 0.64, CI: 0.29–1.42).

Conclusions: The *MIF* -173C allele is associated with erythema nodosum in Löfgren's syndrome, but not with susceptibility to sarcoidosis. This indicates a role for MIF after antigen presenting to the T cell has taken place and the sarcoid inflammatory response has begun.

1. Introduction

Macrophage migration inhibitory factor (MIF) has been shown to be a key regulator in innate and adaptive immune responses. It is constitutively expressed and stored in intracellular pools in a broad variety of cells of the immune system, like T-cells and macrophages [1,2]. MIF is also expressed by cells and tissues, that are in direct contact with the host's natural environment such as the lung [3] and the skin [4]. It is rapidly released after exposure to microbial products and proinflammatory mediators [5].

Once released, MIF contributes to an excessive inflammatory response directly by promoting the production or expression of

Abbreviations: MIF, macrophage migration inhibitory factor; EN, erythema nodosum; BHL, bilateral hilar lymphadenopathy; IPF, idiopathic pulmonary fibrosis; HLA, humon leukocyte antigen; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon; SNP, single nucleotide polymorphism; CI, confidence interval; OR, odds ratio.

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http://dx.doi.org/10.1016/j.cyto.2014.05.020
1043-4666/

pro-inflammatory molecules, like cytokines such as TNF-α, IFN-γ, IL-1β, IL-2, IL-6 and IL-8, and indirectly by antagonizing the anti-inflammatory effects of glucocorticoids [6,7]. The magnitude of this effect varies with the concentration of both glucocorticoids and MIF [8,9].

Recent studies showed an important role for MIF in the pathogenesis of acute and chronic inflammatory and autoimmune diseases [10,11]. Increased levels of MIF have been detected in the synovial fluids of patients with rheumatoid arthritis and serum of patients with juvenile idiopathic arthritis and sarcoidosis [12,13]. Associations between MIF and other pulmonary diseases, like asthma [14], lung cancer [15] and IPF [16], have also been described.

A single nucleotide polymorphism in the 5′ region of the MIF gene, *MIF* -173∗C, is associated with increased MIF protein production, in vivo and in vitro [11]. Associations have been shown between the minor *MIF* -173C allele and rheumatoid arthritis, juvenile idiopathic arthritis, systemic sclerosis [17], systemic lupus erythematosus [18] and sarcoidosis. In sarcoidosis patients with erythema nodosum (EN) the frequency of the *MIF* -173C allele have been found to be significantly higher than in patients with EN due to other causes or controls, indicating a role for MIF in the clinical presentation of sarcoidosis [19].

Sarcoidosis is a multisystemic granulomatous disease of which the cause remains unknown. Several reports support the hypothesis that sarcoidosis results from exposure of genetically susceptible hosts to specific environmental agents, thereby inducing an immune response mediated by macrophages and lymphocytes [20]. Erythema nodosum due to sarcoidosis is usually seen as part of the Löfgren's syndrome, the best defined phenotype of sarcoidosis. Löfgren's syndrome, the acute form of sarcoidosis presents with bilateral hilar lymphadenopathy, erythema nodosum (EN) and/or articular inflammation or arthritis [21]. It is seen in about one third of the sarcoidosis patients and has a good prognosis [22]. Associations have been found between variations in several genes encoding for molecules with important functions in the immune system, such as TNF-a, IFN-g and HLA-molecules [23–25], and Löfgren's syndrome. In particular HLA-DRB1∗03:01 has been strongly associated with Löfgren's syndrome and its outcome [26].

The aim of this study was to investigate whether polymorphisms of the macrophage migration inhibitory factor (MIF) associate with the susceptibility to and the clinical manifestations, arthritis or EN, of Löfgren's syndrome.

2. Materials and methods

2.1. Subjects

A total of 171 unrelated Caucasian patients, from 2 hospitals in the Netherlands, were included in the study. All patients were diagnosed in accordance with the consensus of the ATS/ERS/WASOG statement on sarcoidosis. All patients presented with the classic symptoms of Löfgren's syndrome: acute onset with bilateral hilar lymphadenopathy, fever, erythema nodosum and/or bilateral ankle arthritis.

Presence of EN and arthritis was collected from medical records for all patients.

Three hundred and thirteen healthy Caucasian subjects were included as controls in this study, matched by sex and ethnicity with the Löfgren's syndrome patients.

Written consent was obtained from all subjects, and authorization was given by the Ethics Committee of the St. Antonius Hospital, Nieuwegein and Leiden University Medical Center.

2.2. Genotyping

Genomic DNA was extracted from peripheral blood of each individual using standard methods. We genotyped 171 patients and 313 controls for *MIF* -173G/C (SNP rs755622). Primers were used to amplify a 374 bp PCR product for rs755622 (forward primer: 5′-CTGGCGACTAACATCGGTGA-3′; reverse primer: 5′-ACATCGGCA TGATGGCAGAA-3′), which was digested by AluI restriction enzyme (New England Biolabs) overnight at 37 °C. Restriction product was separated on a 2% agarose gel, the G allele consisted of 2 fragments of 74- and 300-bp, the C allele consisted of 3 fragments of 74-, 94-and 206-bp.

2.3. Statistical analysis

Allele and genotype frequencies were calculated for the -173G to C polymorphism and tested for Hardy–Weinberg equilibrium (HWE) in controls. Case-control association studies were analyzed by X2 test using contingency tables of genotype and allele frequencies. Hardy–Weinberg equilibrium (HWE), Odds ratios and confidence intervals (CI) were calculated with an online tool, available at http://ihg.gsf.de/ihg/snps.html A p value <0.05 was considered significant. The power of the study was calculated using the software Quanto.

A power of >80% was estimated for the analysis between the full set of patients and controls (considering $p = 0.05$ and a reference OR = 1.70) and for the analysis between subtypes of patients (considering $p = 0.05$ and reference OR = 3.3).

3. Results

3.1. Patient characterization

All 171 patients had an acute onset of the disease, with BHL, EN and/or periarticular inflammation or arthritis of the ankles. Thirty-four patients (20%) had only EN, while 43 patients (25%) had only arthritis and 94 patients (55%) had both of the characteristics of the disease. Hundred and six patients (62%) were female. No significant differences were found in clinical manifestations between males and females (see Table 1).

Table 1
Baseline characteristics of controls and patients with Löfgren's syndrome.

		n	%	Sex		Age
				Male	Female	Mean years
Controls		313		118 (37%)	199 (63%)	40
Löfgren's syndrome patients		171		65 (38%)	106 (62%)	35
	EN only	34	20	12 (18%)	22 (21%)	
	Arthritis and EN	94	55	35 (54%)	59 (56%)	
	Arthritis only	43	25	18 (28%)	25 (23%)	

EN only: patients having only erythema nodosum (EN), Arthritis only: patients having only arthritis, Arthritis and EN: patients having both arthritis and EN.