

# Macrophage Migration Inhibitory Factor Gene Polymorphism Is Associated with Sarcoidosis in Biopsy Proven Erythema Nodosum

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**ABSTRACT. Objective.** To assess whether polymorphism of the macrophage migration inhibitory factor (MIF) gene at the position -173 is implicated in the development of sarcoidosis.

**Methods.** Twenty-eight patients with biopsy proven erythema nodosum (EN) associated with sarcoidosis, 70 patients with biopsy proven EN related to other etiologies, and 122 healthy matched controls from the Lugo region of Northwest Spain were studied. Patients and controls were genotyped for a single nucleotide polymorphism in the 5'-flanking region at position -173 of the MIF gene, using SNaPshot ddNTP primer extension, followed by capillary electrophoresis (ABI 3100).

**Results.** A significantly increased frequency of the C mutant allele was observed in patients with EN secondary to sarcoidosis compared to controls ( $p = 0.0016$ ;  $p_{\text{corr}} = 0.0032$ ; OR 2.78, 95% CI 1.45, 5.35) and also compared to patients with EN unrelated to sarcoidosis ( $p = 0.0004$ ;  $p_{\text{corr}} = 0.0008$ ; OR 3.72, 95% CI 1.75, 7.87). Patients with EN carrying an MIF 173 C allele were found to have an increased risk of sarcoidosis (57% in EN secondary to sarcoidosis vs 24% in patients with EN related to other etiologies;  $p = 0.002$ ;  $p_{\text{corr}} = 0.004$ ; OR 4.16, 95% CI 1.64, 10.50).

**Conclusion.** This is the first attempt to assess the influence of MIF genetic polymorphism at position -173 in the development of sarcoidosis. The MIF 173 C allele is associated with a significantly increased risk of developing sarcoidosis in patients with EN. (J Rheumatol 2002;29:1671-73)

*Key Indexing Terms:*  
SARCOIDOSIS

ERYTHEMA NODOSUM

MACROPHAGE MIGRATION INHIBITORY FACTOR GENE

Erythema nodosum (EN) is a self-limiting hypersensitivity reaction characterized by multiple and bilateral inflammatory nodules. They predominantly involve the lower extremities and gradually evolve from painful inflammatory, non-scarring cutaneous and subcutaneous red nodules to a bruised appearance and complete resolution. It may be associated with a wide variety of disease processes including systemic diseases and infections, and use of oral contraceptives and other drugs<sup>1,2</sup>.

Sarcoidosis is a multisystemic disease characterized by tissue infiltration by mononuclear phagocytes with associated granuloma formation<sup>3</sup>. In unselected series of EN, this disease of uncertain etiology usually accounts for 20-30% of cases of EN<sup>2,4</sup>. An important step in the study of sarcoidosis may be the search for genetic markers to differ-

entiate EN associated with sarcoidosis from other conditions presenting with EN. In this regard, we recently examined the immune HLA implications in a series of unselected patients with EN<sup>5</sup>. However, although patients with EN associated with sarcoidosis had an increased frequency of HLA-DRB1\*13 and HLA-DRB1\*03 compared with controls, no significant differences between the subgroup of sarcoidosis and the rest of the patients with EN were found<sup>5</sup>.

Culture supernatants of cutaneous granulomas from patients with sarcoidosis contain macrophage migration inhibitory factor (MIF)<sup>6</sup>. This is a predominantly macrophage derived cytokine associated with a number of proinflammatory actions. Thus, it induces secretion of tumor necrosis factor alpha (TNF- $\alpha$ ), promotes interferon- $\gamma$  induced production of nitric oxide, and has been associated with the development of tuberculin delayed-type hypersensitivity reaction<sup>7,8</sup>. Due to its role in TNF- $\alpha$  synthesis and T cell activation MIF has been implicated in the pathogenesis of rheumatoid arthritis (RA)<sup>9</sup>. In both RA and animal models of arthritis, MIF has been identified as a key upstream regulator of local inflammatory profile by induction of TNF- $\alpha$  production<sup>9,10</sup>. Of note, a biallelic polymorphism (G to C transition) in the 5'-flanking region at position -173 of the MIF gene has been associated with systemic onset juvenile idiopathic arthritis<sup>11</sup>. This polymorphism (MIF 173 G to C) creates an AP-4 transcription factor

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binding site and may be of functional significance. We examined whether the polymorphism of this gene is also implicated in EN associated with sarcoidosis.

## MATERIALS AND METHODS

**Patients.** Clinical data for most of the patients included in this study have been reported<sup>4,5</sup>. Briefly, all patients (n = 98, aged 15–78 years) were diagnosed with biopsy proven EN in close collaboration between the Rheumatology and Dermatology Divisions of Hospital Xeral-Calde. Twenty-eight were diagnosed with sarcoidosis. The remaining 70 patients were diagnosed with idiopathic EN (when no underlying diseases or precipitating events were found; n = 36) or developed EN generally in the context of an infectious disease, drug intake, and more rarely in the setting of an inflammatory bowel disease or Sweet's syndrome. Patients and ethnically matched controls were from the Lugo region in Galicia (Northwest Spain).

**Histopathological analysis.** As described<sup>4</sup>, patients were included in this study if they had a skin biopsy showing an acute or granulomatous septal panniculitis with primary inflammation around the veins of the septal system involving neutrophils, lymphoid cells, and histiocytes, with or without giant cell formation. In some instances histologic lesions had the appearance of a granulomatous inflammatory infiltrate that typically spared the fat lobule except by contiguous spread.

EN secondary to sarcoidosis was defined when sterile noncaseating granulomas were obtained in tissue biopsies<sup>4</sup>. However, in those cases presenting with typical Löfgren's syndrome (EN and bilateral hilar adenopathy with or without peripheral arthritis), the presence of a tissue biopsy showing noncaseating granulomas was not required if after a followup of at least one year there was no other condition responsible for the onset of EN.

**Genotyping.** We used SNaShot ddNTP primer extension kit for genotyping the -173 G to C polymorphism of the MIF. DNA from patients and controls was extracted from anticoagulated blood collected in EDTA using a commercial DNA extraction kit (Bioline<sup>TM</sup>). The following primers were used for PCR amplification: forward 5'-ACT AAG AAA GAC CCG AGG C-3'; reverse 5'-GGG GCA CGT TGG TGT TTA C-3'. A total of 20 ng genomic DNA was amplified in a 10 µl final PCR reaction volume containing 5 pmoles of each primer, 0.08 nmoles of dNTPs, 10× KCl buffer, and 0.6 units of Taq polymerase (Bioline). All the reactions were performed in 384 well microtiter plates on a Tetrad thermal cycler (MJ Research, Waltham, MA, USA). The DNA was denatured at 95°C for 5 min followed by 40 cycles of 95°C for 45 s, 60°C for 45 s, and 72°C for 45 s. The final extension was carried out at 72°C for 5 min.

The presence of the 364 bp PCR product was verified on a 2% agarose gel stained with ethidium bromide. The probe used for the single nucleotide extension in the primer extension kit was 5'-AGC CGC CAA GTG GAG AAC AG-3'. After extension and purification, the product was electrophoresed on a 3100 ABI analyzer and the results were analyzed with Genescan software.

**Statistical analysis.** Strength of association between patient groups and controls and alleles or genotypes of the -173 G to C polymorphism was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined using contingency tables by either chi-square or Fisher's exact analysis. Statistical significance was defined as p ≤ 0.05. Corrected p values (p<sub>corr</sub>) by Bonferroni correction were shown. Calculations were performed with the statistical package Stata V6.

## RESULTS

No evidence of departure from Hardy-Weinberg equilibrium was observed in the control group (p > 0.05).

A significantly increased frequency of the mutant allele C was observed in patients with EN secondary to

sarcoidosis compared with controls (p = 0.0016; p<sub>corr</sub> = 0.0032; OR 2.78, 95% CI 1.45, 5.35). In contrast, there was no increase in the mutant MIF 173 C in the remaining patients with EN. Thus, a significant difference in the frequency of MIF 173 C between EN secondary to sarcoidosis and the group of EN secondary to other etiologies was also observed (p = 0.0004; p<sub>corr</sub> = 0.0008; OR 3.72, 95% CI 1.75, 7.87) (Table 1).

Only one MIF 173 CC and no MIF 173 CC homozygote was found in the control group and in the group of EN unrelated to sarcoidosis, respectively (Table 2). In contrast, 3 (11%) of the patients with EN secondary to sarcoidosis had this genotype. The frequency of the 3 possible genotypes differed significantly between patients with EN secondary to sarcoidosis and controls (p = 0.002). This was also the case when the distribution of genotypes in patients with sarcoidosis was compared with that observed in patients with EN due to other etiologies (p = 0.001). This was due to an increase in both heterozygous and homozygous mutants being observed in patients with EN secondary to sarcoidosis (Table 2).

Finally, patients with EN having a MIF 173 C were found to have an increased risk of sarcoidosis (57% in EN secondary to sarcoidosis vs 24% in patients with EN related

Table 1. Allele frequencies of MIF polymorphism in a series of patients with biopsy proven EN and controls.

	Controls, n = 122	EN Associated with Sarcoidosis, n = 28	EN Related to Other Etiologies, n = 70
Allele (2N)			
G (%)	206 (85)	37 (66)	123 (88)
C (%)	38 (15)*	19 (34)*,**	17 (12)**

Allele C was significantly increased in EN secondary to sarcoidosis compared with controls\* and EN secondary to other etiologies\*\*. \*p = 0.0016; p<sub>corr</sub> = 0.0032; OR 2.78, 95% CI 1.45, 5.35; \*\* p = 0.0004; p<sub>corr</sub> = 0.0008; OR 3.72, 95% CI 1.75, 7.87.

Table 2. Genotype distribution of MIF polymorphism in a series of patients with biopsy proven EN and controls.

	Controls*, n = 122	EN Associated with Sarcoidosis*,**, n = 28	EN Related to Other Etiologies**, n = 70
Genotype			
GG (%)	85 (70)	12 (43)	53 (76)
GC (%)	36 (29)	13 (46)	17 (24)
CC (%)	1 (1)	3 (11)	0 (0)

\* EN secondary to sarcoidosis compared with controls: p = 0.002; \*\* EN secondary to sarcoidosis compared with EN related to other etiologies: p = 0.001. Distribution of GC + CC genotypes versus GG genotype: in patients with EN secondary to sarcoidosis compared with controls: p = 0.007; p<sub>corr</sub> 0.014; OR 3.06, 95% CI 1.32, 7.11. In patients with EN secondary to sarcoidosis compared with EN due to other etiologies: p = 0.002; p<sub>corr</sub> = 0.004; OR 4.16, 95% CI 1.64, 10.50.

to other etiologies;  $p = 0.002$ ;  $p_{\text{corr}} = 0.004$ ; OR 4.16, 95% CI 1.64, 10.50).

## DISCUSSION

Macrophage MIF is an immunoregulatory cytokine essential for T cell activation and delayed type hypersensitivity reaction<sup>12</sup>. A potential role for MIF is suggested in many inflammatory and autoimmune conditions<sup>13</sup> and early studies suggested that serum levels of MIF are elevated in patients with sarcoidosis<sup>14</sup>. At present there is considerable evidence supporting the concept of sarcoidosis being the consequence of an antigen driven cell mediated immune response in the context of innate immunity activation resulting in a delayed-type hypersensitivity immune reaction and granuloma formation<sup>15</sup>. Restricted use of T cell receptor and association of HLA phenotype in patients with Löfgren's syndrome suggest the contribution of an antigen-specific immune reaction in sarcoidosis<sup>16</sup>. Cellular interactions contributing to granuloma formation in sarcoidosis are not very well characterized and several cytokines and chemokines seem to be participating in this process<sup>17</sup>.

Two robust lines of evidence support a genetic component in the pathogenesis of sarcoidosis: racial variation in its epidemiology and familial clustering of cases<sup>18</sup>.

Our study is the first to assess the influence of the MIF gene in the development of sarcoidosis. Unlike other patients presenting with EN, those with EN in the setting of this granulomatous disease exhibited an increased frequency of mutant MIF 173 C allele.

MIF is released from macrophages and T cells in response to physiologic concentrations of glucocorticoids<sup>19</sup>. This is in contradiction with the role of MIF as a proinflammatory cytokine, but supports the concept that physiologic levels of glucocorticoids regulate the immune inflammatory response. MIF also acts in a dose dependent manner in regulating the inhibitory effects of glucocorticoids in the immune system<sup>13</sup>. MIF is inhibited by pharmacological concentrations of glucocorticoids. However, at low concentrations these drugs increase the synthesis of MIF<sup>9</sup>.

Unlike in patients with progressive pulmonary impairment, noncaseating granulomatous skin lesions, or visceral involvement, treatment with glucocorticoids is not generally required in most cases of EN associated with sarcoidosis, where spontaneous resolution is frequent<sup>4</sup>. However, the use of these drugs in patients with EN yields a rapid improvement of the cutaneous nodules. Thus, it is possible that the genetic polymorphism in MIF may be implicated in an impaired chronic inflammatory response in patients with sarcoidosis.

These observations may have biological implications. They would contribute to the existing knowledge in terms of stratification of EN as well as understanding the contribution of MIF polymorphism to immune disease susceptibility.

Studies in patients with chronic sarcoidosis in particular

and in other populations frequently associated with severe visceral involvement are required to further assess the pathologic role of MIF gene polymorphism.

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