

Lack of association between macrophage migration inhibitory factor gene (-173 G/C) polymorphism and cutaneous vasculitis

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Received on February 9, 2006; accepted on April 14, 2006.

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Key words: Cytokines, macrophages, macrophage migration inhibitory factor gene, cutaneous vasculitis, Henoch-Schönlein purpura, cutaneous leukocytoclastic angiitis.

ABSTRACT

Objective. To assess whether polymorphism of the macrophage migration inhibitory factor (MIF) gene at position -173 was implicated in the incidence of Henoch-Schönlein purpura (HSP) and cutaneous leukocytoclastic angiitis (CLA). A further objective was to determine if any relationship existed with severe systemic complications of HSP, in particular with severe renal involvement and permanent renal dysfunction.

Methods. Unselected patients from Northwest Spain with primary cutaneous vasculitis classified as HSP or hypersensitivity vasculitis (HV) according to proposed criteria were studied. Patients with HV were included in this study if they fulfilled the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis definitions for CLA. Patients and controls were genotyped for a single nucleotide polymorphism (SNP) in the 5'-flanking region at position -173 of the MIF gene, using SNaPshot ddNTP primer extension, followed by capillary electrophoresis (ABI 3100).

Results. Ninety-five Caucasian patients (57 classified as having HSP and 38 who fulfilled definitions for CLA) and 122 healthy controls were studied. No allele or genotype differences between the whole group of HSP or CLA patients and controls were observed. This was also the case when HSP patients were stratified by the presence of gastrointestinal complications, nephritis, and permanent renal involvement (renal sequelae).

Conclusions. The polymorphism in MIF gene promoter (-173 G/C) does not appear to be genetic risk factors for cutaneous vasculitis in Northwest Spain.

Introduction

Henoch-Schönlein purpura (HSP) and cutaneous leukocytoclastic angiitis (CLA) are the most common primary small-sized blood vasculitides (1). Both conditions are characterized by infiltration of the small blood vessels with polymorphonuclear leukocytes and the presence of leukocytoclasia (2, 3). However, in HSP IgA-dominant

immune deposits in the walls of the small vessels and in the renal glomeruli are frequently observed (2, 3). HSP is common in children and rare in adults (1). Palpable purpura, joint and gastrointestinal manifestations are typical of this condition. Renal manifestations constitute the most serious complications and long-term morbidity and mortality in HSP are mainly due to renal involvement (1). CLA is an isolated vasculitis limited to skin (3, 4). Thus, for the diagnosis of CLA, systemic involvement must be excluded (3). In patients with CLA a recent history of exposure to drugs or infections is often present (4).

Susceptibility to HSP and CLA and associated clinical heterogeneity in HSP may be conferred by a number of genetic loci. Previous studies in patients with cutaneous vasculitis from Northwestern Spanish have shown that different genes may influence the phenotype and the outcome of HSP (5-10).

Macrophage migration inhibitory factor (MIF) is an immunoregulatory cytokine, originally identified as a T cell derived factor. MIF is a potent activator of macrophages, inhibits the random migration of macrophages, concentrating macrophages at the inflammatory site and it is thought to play an important role in cell mediated immunity (11, 12).

Elevation of MIF serum level has been reported in various inflammatory and autoimmune diseases. Interestingly, in patients with Wegener's granulomatosis, a necrotizing vasculitis affecting small to medium sized blood vessels, high serum MIF levels have been described (13). Also, a positive MIF test towards a drug was reported in a case of cutaneous vasculitis (14). Although no information about serum MIF levels in patients with HSP has been reported, monocytes/macrophages are the predominant cell type infiltrating glomeruli in HSP nephritis (15). In this regard, the excess of monocytes and T cell subsets in the glomeruli of HSP patients with crescentic proliferative nephritis suggests a potential role of cellular macrophages in the pathogenesis of HSP nephritis (16).

Association between functional poly-

morphisms in the promoter region of the MIF gene has been reported in both juvenile and adult rheumatoid arthritis (17-19). This suggests that the overproduction of MIF observed in these inflammatory conditions is as a consequence of genetically predetermined dysregulated (excessive) MIF production. We have previously reported an association of the MIF gene -173 (G/C) polymorphism in patients with erythema nodosum secondary to sarcoidosis (20). Since sarcoidosis is an inflammatory disease characterized by tissue infiltration of mononuclear phagocytes, we have further investigated the potential role of the MIF -173 (G/C) polymorphism in a series of unselected patients with cutaneous vasculitis and, especially in the susceptibility to HSP nephritis.

Patients and methods

Patients were recruited from the Divisions of Pediatrics and Rheumatology of the Hospital Xeral-Calde (Lugo, Northwest Spain). Controls, matched by ethnicity, age and sex, were also obtained from the same geographic area. Patients and controls were included in this study after written informed con-

sent. We obtained approval for the study from the local ethical Committee.

Inclusion criteria.

Patients with primary cutaneous vasculitis who fulfilled the 1990 American College of Rheumatology classification criteria for hypersensitivity vasculitis (HV) or HSP (21,22) were differentiated using the criteria proposed by Michel *et al* (23). As previously reported (8), besides patients with HSP, only those HV patients that fulfilled the Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitis definitions for CLA (3) were included in the present study.

All patients were required to have had at least a 2 year follow-up.

MIF genotyping

DNA from patients with cutaneous vasculitis and controls was extracted from anticoagulated blood collected in EDTA using a commercially available DNA extraction kit (Bioline™, London UK). The SNaShot ddNTP primer extension method was used for genotyping the -173 G/C polymorphism of the MIF gene, as previously described (20).

Statistical analysis

Strength of association between patient groups and controls and alleles or genotypes of the MIF -173 (G/C) polymorphism was estimated using odds ratios and 95% confidence intervals. Levels of significance were determined using contingency tables by either Chi-square or Fisher exact analysis. Statistical significance was defined as *p* equal or less than 0.05. Calculations were performed with the statistical package Stata V6.

Results

Ninety-five Caucasian patients (57 classified as having HSP and 38 who fulfilled definitions for CLA) and 122 controls were studied.

The main epidemiological and clinical data of the patients with HSP and CLA are shown in Table I. Hematuria with or without proteinuria and severe gastrointestinal manifestations were frequently observed in the group of patients with HSP. However, after a minimum of a 2 year follow-up (median 8 years) only 12 of the 57 patients had persistent renal involvement (renal sequelae), mainly hematuria.

Patients with CLA presented with maculo-papular or purpuric skin lesions. Drug intake (generally analgesics or antibiotics) within a week prior to the onset of the vasculitis was observed in a third of the cases. Apart from joint manifestations (generally arthralgia) during the course of the vasculitis, no systemic manifestations were observed in these 38 patients after a minimum of a 2-year follow-up (Table I).

In controls no evidence of departure from Hardy-Weinberg equilibrium was found (*p* = N.S.).

No significant differences for the MIF gene polymorphism were observed in patients with cutaneous vasculitis compared to the controls. Allele and genotype frequencies for MIF polymorphism in patients with HSP and CLA and controls are shown in Table II.

The allele and genotype frequencies were also examined in HSP patients stratified by the presence of nephritis or renal sequelae during the course of the disease. However, no statistically significant differences between HSP

Table I. Main features of a series of patients with primary cutaneous vasculitis.

	HSP (N = 57)	CLA (N = 38)
Children (age less than 21 years)/ adults	45/12	2/36
Male/female	29/28	21/17
Age at the onset of the disease (years)		
Median	6	56.5
Range	2- 62	17-77
Duration of follow-up (years)		
Median:	8	4
Palpable purpura and/or maculopapular rash	57 (100%)	38 (100%)
Arthralgia and/or arthritis	39 (68%)	9 (24%)
Gastrointestinal bleeding	24 (42%)	----
Bowel angina	43 (75%)	----
Renal manifestations		
Hematuria	37 (65%)	----
Proteinuria	19 (33%)	----
Nephrotic syndrome	7 (12%)	----
Renal insufficiency	2 (4%)	----
Renal sequelae (persistent renal involvement)	12 (21%)	----

HSP: Henoch-Schönlein purpura.

CLA: Cutaneous leukocytoclastic angiitis.

Table II. Genotype and allele distribution of the MIF -173 (G/C) polymorphism in patients with cutaneous vasculitis (CLA or HSP) and controls.*

		Controls	CLA	HSP	HSP with renal manifestations		HSP with renal sequelae	
		(N = 122)	(N = 38)	(N = 57)	Yes (N = 37)	No (N = 20)	Yes (N = 12)	No (N = 45)
Genotype	GG	85 (70%)	25 (66%)	44 (77%)	28 (76%)	16 (80%)	11 (92%)	33 (73%)
	GC	36 (29%)	12 (31%)	11 (19%)	7 (19%)	4 (20%)	1 (8%)	10 (22%)
	CC	1 (1%)	1 (3%)	2 (4%)	2 (5%)	0 (0%)	0 (0%)	2 (5%)
Allele (2N)	G	206 (84%)	62 (82%)	99 (87%)	63 (85%)	36 (90%)	23 (96%)	76 (84%)
	C	38 (16%)	14 (18%)	15 (13%)	11 (15%)	4 (10%)	1 (4%)	14 (16%)

* No statistically significant differences between patients with cutaneous vasculitis and controls were observed.

patients with or without renal manifestations or between patients with HSP nephritis and those with CLA or controls were observed Table II. This was also the case when HSP patients with severe gastrointestinal complications were compared with those without these manifestations (data not shown).

Discussion

The present report constitutes the first study aimed to assess the potential implication of the MIF gene -173 (G/C) polymorphism in the susceptibility to patients with cutaneous vasculitis who fulfilled classification criteria for HSP or definitions for CLA. However, as observed in patients with giant cell arteritis (24), our results do not support the role of this polymorphism in the susceptibility to and severity of cutaneous vasculitis in Northwestern Spain. Different pathogenic mechanisms between vasculitides such as giant cell arteritis or cutaneous vasculitis and other autoimmune diseases may explain the different results in terms of association of the MIF -173 (G/C) polymorphism. In addition, the different ethnic origins of the world populations may explain differences in terms of genetic susceptibility to autoimmune diseases. This might imply possible different pathogenic mechanisms for the development of cutaneous vasculitis, and in particular of HSP, in different populations. Due to this, additional studies in patients with different genetic backgrounds are required to fully eliminate the potential role of this poly-

morphism in the pathogenesis of HSP and CLA.

In Northwestern Spain, some gene polymorphisms have been associated with disease susceptibility to HSP (5), while others seem to play a specific implication in disease severity (6-10). Likewise, other gene polymorphisms associated with susceptibility to other vasculitides (25) were not found to be implicated in the development or severity of HSP in Northwestern Spain (26).

Macrophages and T cells secrete MIF in response to low physiologic steroid concentration. It has been suggested that a role of MIF is to counter-regulate glucocorticoid effects (27). A potential role for MIF as a counter regulator of glucocorticoid action has been implicated in many inflammatory and autoimmune conditions, and because of that MIF has been considered as a potential candidate gene for susceptibility to autoimmune inflammatory disorders (28).

Strong evidence supports a role of pro-inflammatory monocyte/macrophage cells in the pathogenesis of HSP nephritis (29). However, in contrast to patients with erythema nodosum associated with sarcoidosis (20), a disease also characterized by tissue infiltration of mononuclear phagocytes, our results show that the polymorphism in MIF gene promoter (-173 G/C) is not associated with HSP susceptibility or severity. Thus, other different pathogenic mechanisms may be implicated in the development of the inflammatory disease in this vasculitis.

It is possible that the MIF gene may be implicated in an impaired inflammatory response in patients with sarcoidosis and other inflammatory conditions such as rheumatoid arthritis and juvenile idiopathic arthritis, which show association with MIF gene polymorphisms (17-19,30). However, this does not seem to be the case in HSP or CLA. Further studies to assess the role of the MIF gene -173 (G/C) polymorphism in patients with CLA and HSP with different genetic background are needed to definitively exclude the role of this polymorphism in the pathogenesis of cutaneous vasculitides.

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