

E-selectin polymorphism in erythema nodosum secondary to sarcoidosis

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Received on August 25, 2003; accepted in revised form on December 18, 2003.

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Key words: Sarcoidosis, erythema nodosum, A561C E-selectin gene.

ABSTRACT

Objective. *E-selectin is expressed on cytokine-stimulated endothelial cells and plays an important role in leukocyte-endothelium interactions and in inflammatory cell recruitment. An A/C polymorphism at position +561 in the E-selectin gene, which yields an amino acid exchange from serine to arginine at position 128 in the epidermal growth factor-like domain, has been described. We have assessed whether this bi-allelic polymorphism may be implicated in the clinical expression of erythema nodosum (EN) secondary to sarcoidosis.*

Methods. *Thirty-one patients with biopsy-proven erythema nodosum (EN) associated with sarcoidosis, 68 patients with biopsy-proven EN related to other etiologies and 66 healthy matched controls from the Lugo region of Northwest Spain were studied. Patients and controls were genotyped for the A/C polymorphism gene by PCR-restriction fragment length polymorphism.*

Results. *A significantly reduced frequency of the C mutant allele was observed in patients with EN secondary to sarcoidosis compared to controls ($p = 0.019$) and also compared to patients with EN unrelated to sarcoidosis ($p = 0.028$). 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 ($p = 0.028$) and controls ($p = 0.037$). This was due to an absence in both C/A heterozygotes and C/C homozygotes in patients with EN secondary to sarcoidosis.*

Conclusions. *The present study constitutes the first attempt to assess the influence of E-selectin polymorphism at position +561 in the development of sarcoidosis. The C allele at the +561 position of the E-selectin gene is associated with significantly reduced risk of developing sarcoidosis in patients with EN.*

Introduction

Erythema nodosum (EN) is a self-limiting hypersensitivity reaction characterized by multiple and bilateral inflammatory nodules (1,2). It may be associated with a wide variety of disease processes including systemic diseases, in-

fections, oral contraceptives and other drugs (1, 2).

Sarcoidosis is a systemic disease characterized by tissue infiltration of mononuclear phagocytes with associated granuloma formation (3). In unselected series sarcoidosis usually accounts for 20-30% of cases of EN (2, 4).

The recruitment of cells at sites of inflammatory activity is thought to result from a sequence of events involving rolling, attachment and migration of the inflammatory cells from the bloodstream to the vessel wall. E-selectin (Endothelial leukocyte adhesion molecule-1) is a member of the selectin family of adhesion molecules and is a 115 kDa single chain glycoprotein (5), which is mapped to chromosome 1q23-q25 (6). E-selectin is expressed by cytokine activated endothelial cells (6). Studies have suggested its role in the adhesion of neutrophils, monocytes, T lymphocytes, eosinophils and basophils (7). High levels of E-selectin have been reported in serum of patients with active sarcoidosis compared with controls (8).

An A/C polymorphism at position +561 in the E-selectin gene, which yields an amino acid exchange from serine to arginine at position 128 (S128R) in the epidermal growth factor like-domain, has been described (9). This polymorphism has been associated with a higher risk for early severe atherosclerosis and coronary artery disease (9, 10). An association with this polymorphism has also been observed in systemic lupus erythematosus (SLE) (11).

In the present study we have examined the E-selectin S128R implication in sarcoidosis associated with EN.

Patients and methods

Patients and controls

Clinical data of the patients included in this study have been previously reported (4,12). Thirty-one were diagnosed with sarcoidosis. The remaining 68 patients were diagnosed as having idiopathic EN (when no underlying diseases or precipitating events were found; $n = 35$) or developed EN generally in the context of an infectious disease or drug-intake. As previously reported (12), either idiopathic EN or secondary to an

underlying condition, was more common in women. However, patients with EN secondary to sarcoidosis were generally younger than those with idiopathic EN (44.4 ± 15.9 versus 46.1 ± 15.6 years). For the purpose of this study a group of sex and ethnically matched controls ($n=66$) with a mean age of 45 years was assessed. Patients and controls were from the Lugo region of Northwest Spain.

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 containing neutrophils, lymphoid cells, and histiocytes, with or without giant cell formation (1, 4).

EN secondary to sarcoidosis was defined when sterile non-caseating granulomas were obtained in tissue biopsies. 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 for diagnosis of sarcoidosis if after a follow-up of at least 1 year there was no other condition responsible for the occurrence of EN (4).

Genotyping

Patients and controls were genotyped for the A/C polymorphism gene by PCR-restriction fragment length polymorphism as previously reported (11).

Statistical analysis

The association between patient groups and controls and alleles or genotypes of A/C polymorphism was estimated using odds ratios (OR) and 95% confidence intervals (CI). Levels of significance were determined by either Chi-square or Fisher exact analysis. Statistical significance was defined as $p < 0.05$.

Results

In controls no evidence of departure from Hardy-Weinberg equilibrium was observed for this polymorphism.

No statistically significant differences between the whole group of biopsy-proven EN and controls were found for

the E-selectin +561 polymorphism (Table I). However, when patients with EN were stratified according to their underlying disease some differences were seen. In this regard, none of the 31 biopsy-proven EN secondary to sarcoidosis exhibited the allele C. In contrast, 10 of the 66 controls and 10 of the 68 patients with EN unrelated to sarcoidosis carried this allele. A significantly reduced frequency of the allele C was observed in patients with EN secondary to sarcoidosis compared with controls ($p=0.019$; OR: 0 [95% CI 0.0 – 0.7]) and also compared to patients with EN unrelated to sarcoidosis ($p=0.028$; OR: 0 [95% CI 0.0– 0.8]) (Table I).

The frequency distribution of the 3 possible genotypes differed significantly between patients with EN secondary to sarcoidosis and controls ($p=0.037$). 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 ($p=0.028$). This was due to an absence in both heterozygote and homozygote mutants in patients with EN secondary to sarcoidosis (Table I). Finally, EN patients with the AA genotype were found to have an increased risk of sarcoidosis (100% in EN secondary to sarcoidosis versus 85% in

patients with EN related to other etiologies; $p=0.028$).

Discussion

Susceptibility to sarcoidosis and associated clinical heterogeneity in this condition may be conferred by a number of genetic loci. We have previously shown that the polymorphism of the macrophage migration inhibitory factor gene at position –173 is implicated in the development of EN secondary to sarcoidosis (13).

It is likely that expression of adhesion molecules plays a role in this mechanism of accumulation of inflammatory cells in the lung in patients with sarcoidosis. Cytokine-inducible regulatory sequences such as NF- κ B and AP-1 binding sites are presented in the human E-selectin gene (6) and induction and expression of E-selectin has been described for cultured endothelial cells exposed to IL-1, TNF or bacterial endotoxin (7). Cronstein *et al.* suggested that the anti-inflammatory effects of corticosteroids could be involved in mechanisms that block the expression of E-selectin (14). The use of these drugs in patients with EN yields a rapid improvement of the cutaneous nodules (4). *In vitro* studies have suggested that the S128R polymorphism alters E-selectin function, which raises the pos-

Table I. Allele frequencies and genotype distribution of E-selectin +561 single nucleotide polymorphism in a series of 99 patients with biopsy-proven erythema nodosum (EN) and 66 ethnically matched controls.

No. cases	Controls (N=66)	EN Total (N=99)	EN secondary to sarcoidosis (N=31)	Other EN (N=68)
Allele (2N)				
A	121 (92%)	188 (95%)	62 (100%)	126 (93%)
C	11 (8%) ^a	10 (5%)	0 (0%) ^{a,b}	10 (7%) ^b
Genotype				
AA	56 (85%)	89 (90%)	31 (100%)	58 (85%)
AC	9 (14%)	10 (10%)	0 (0%)	10 (15%)
CC	1 (1%)	0 (0%)	0 (0%)	0 (0%)

^a EN secondary to sarcoidosis compared to controls ($p=0.019$; OR: 0 [95% CI 0.0 – 0.7])

^b EN secondary to sarcoidosis compared to EN related to other etiologies ($p=0.028$; OR: 0 [95% CI 0.0 – 0.8])

Genotype distribution in EN secondary to sarcoidosis compared with controls: $p=0.037$

Genotype distribution in sarcoidosis compared with EN related to other etiologies: $p=0.028$

Distribution of AA genotypes versus AC + CC genotype:

In patients with EN secondary to sarcoidosis compared with controls: $p=0.028$

In patients with EN secondary to sarcoidosis compared with EN due to other etiologies: $p=0.028$

sibility that this may critically modify leukocyte-endothelial cell interaction predisposing to inflammatory vascular disease (15,16).

An increase in mutant allele C was observed in UK and Spanish SLE patients (OR 1.7 and 1.8, respectively) compared with ethnically matched controls (11). This condition is associated with a higher risk of developing atherosclerosis. Also, an increase in allele C was observed in patients with severe atherosclerosis (15.5%) compared with an unselected population (8.8%) (9). In addition, the increase of C allele was found in 16 of 82 (19.5%) patients with angiographically documented coronary artery disease compared with only 10.6% in a series of 71 normal controls (10). In contrast, our study shows that none of the patients with EN associated with sarcoidosis carried the allele C and a significant increase in allele A was observed compared with controls. This could be due to the functional implication of this polymorphism in the development of sarcoidosis being different from the pathogenic mechanism involved in other conditions such as SLE and atherosclerosis, regardless of the inflammatory burden observed in all these conditions. This fact may suggest that our patients may have a lower risk of developing severe atherosclerotic disease.

The way in which the A/C polymorphism may predispose to EN associated with sarcoidosis is unknown. There are two possible explanations: either the A allele may predispose to disease devel-

opment or the C mutant allele may yield protection against EN secondary to sarcoidosis. Alternatively, this polymorphism may not be directly implicated in the disease susceptibility and it may be in linkage disequilibrium with another polymorphism in the vicinity of the E-selectin gene.

The potential contribution of sarcoidosis itself, regardless of the association with EN, as the main factor for the results observed in this study should not be ignored. Due to this, additional studies in sarcoidosis patients without EN will be required to confirm the role of E-selectin gene polymorphism in the development of this disease.

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