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Endothelial nitric oxide synthase polymorphisms in biopsy-proven erythema nodosum from a defined population

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ABSTRACT

Objective. *To assess the potential influence of endothelial nitric oxide synthase (eNOS) polymorphisms in the susceptibility to and clinical expression of a series of patients diagnosed with biopsy-proven erythema nodosum (EN).*

Methods. *Ninety-seven unselected patients from Northwest Spain with biopsy-proven EN were studied. Patients and ethnically matched controls were genotyped by PCR based techniques for a variable number tandem repeat polymorphism in intron 4, a T/C polymorphism at position -786 in the promoter region and a polymorphism in exon 7 (298Glu/Asp or 5557G/T) of the eNOS gene.*

Results. *No differences in allele or genotype frequencies for any of the individual eNOS polymorphisms were observed between biopsy-proven patients with EN and controls. It was also the case when patients with EN secondary to sarcoidosis were compared with the remaining patients or controls. In the group of patients with EN, no linkage disequilibrium between these polymorphisms was found. Also, no significant differences in haplotype frequencies were observed between patients and controls.*

Conclusions. *Our present results do not support a role for eNOS polymorphisms in the susceptibility to and clinical expression of EN.*

Introduction

Erythema nodosum (EN), the most frequent type of the panniculitis, is a self-limiting hypersensitivity cutaneous reaction consisting of inflammatory, tender, warm nodular lesions, usually bilaterally distributed and located on the anterior aspects of the shins, ankles and knees (1, 2). It may be idiopathic, secondary to a wide variety of diseases, such as infections, sarcoidosis, inflammatory bowel diseases, connective tissue and other autoimmune disorders and malignancies, observed during pregnancy or associated to different medications (1, 2).

Nitric oxide (NO) has been implicated in the pathogenesis of autoimmune rheumatic diseases such as rheumatoid arthritis (3). This is produced constitutively by endothelial (eNOS or NOS3), or neuronal synthases (nNOS or NOS1), or in higher concentrations by iNOS (or NOS2) after stimulation of a variety of pro-inflammatory cytokines (4). Several functional relevant polymorphisms in the eNOS gene have been associated with the development of primary (5, 6) or secondary vasculitides (7) in different populations. On these bases, although EN is not properly a vasculitis but the stereotypical example of a mostly septal panniculitis, in the present study we aimed to assess whether eNOS polymorphisms might be implicated in the pathogenesis of EN. Moreover, since we have previously observed some immunogenetic differences between biopsy-proven EN associated to sarcoidosis and those idiopathic or associated to conditions different from sarcoidosis (8, 9), we also studied whether potential differences in these polymorphisms might be useful to discriminate patients with EN associated to sarcoidosis from other patients with EN.

Patients and methods

Patients and controls

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 constituted by neutrophils, lymphoid cells, and histiocytes, with or without giant cell formation (1, 10).

Clinical data of the patients included in this study have previously been reported (10, 11). Briefly, all of them (n = 97; 81 women; mean age [range]: 44 [15-78] years) were diagnosed with biopsy-proven EN in close collaboration between the Rheumatology and Dermatology Divisions of the Hospital-Xeral Calde, Lugo (Spain). Thirty-five patients were diagnosed as having idiopathic EN (when no underlying diseases or precipitating events were found). The remaining 62 patients were diagnosed with EN secondary to sarcoidosis (n = 30) 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 syn-

Table I. Allele and genotype frequencies of eNOS gene polymorphisms in patients with biopsy-proven erythema nodosum and controls.*

Gene	Controls	Erythema nodosum			
		Total	Idiopathic	Secondary	With sarcoidosis
eNOS (intron 4)	(n = 118)	(n = 94)	(n = 32)	(n = 62)	(n = 30)
Genotype					
11	85 (72%)	65 (69%)	19 (59%)	46 (74%)	22 (73%)
12	31 (26%)	29 (31%)	13 (41%)	16 (26%)	8 (27%)
22	2 (2%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Allele					
1	201 (85%)	159 (85%)	51 (80%)	108 (87%)	52 (87%)
2	35 (15%)	29 (15%)	13 (20%)	16 (13%)	8 (13%)
eNOS (exon 7)	(n = 97)	(n = 83)	(n = 28)	(n = 55)	(n = 27)
Genotype					
GG	35 (36%)	22 (26%)	9 (32%)	13 (24%)	4 (15%)
GT	45 (46%)	47 (57%)	15 (54%)	32 (58%)	19 (70%)
TT	17 (18%)	14 (17%)	4 (14%)	10 (18%)	4 (15%)
Allele					
G	115 (59%)	91 (55%)	33 (59%)	58 (53%)	27 (50%)
T	79 (41%)	75 (45%)	23 (41%)	52 (47%)	27 (50%)
eNOS (-786)	(n = 117)	(n = 95)	(n = 35)	(n = 60)	(n = 29)
Genotype					
TT	37 (32%)	29 (31%)	13 (37%)	16 (27%)	8 (28%)
TC	58 (50%)	45 (47%)	16 (46%)	29 (48%)	12 (41%)
CC	22 (19%)	21 (22%)	6 (17%)	15 (25%)	9 (31%)
Allele					
T	132 (56%)	103 (54%)	42 (60%)	61 (51%)	28 (48%)
C	102 (44%)	87 (46%)	28 (40%)	59 (49%)	30 (52%)

*No statistically significant differences between the different groups were observed.

drome. Patients and matched controls (n = 118) were from the Lugo region in Galicia (Northwestern Spain). Information on the characteristics of this well defined, stable and ethnically homogenous, mixed rural and urban, white population living in the region of Lugo, central Galicia, has been described elsewhere (12-14).

EN secondary to sarcoidosis was defined when sterile noncaseating 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 if after a follow-up of at least 1 year there was no other condition responsible for the occurrence of EN (8-11).

Informed consent was obtained to perform this study.

Genotyping

DNA from patients with EN and controls was extracted from anticoagulated blood collected in EDTA using a commercially available DNA extraction kit (Bioline™, London UK). As previously described (6, 15), patients and controls were genotyped by PCR based techniques for a variable number tandem repeat polymorphism in intron 4, a T/C polymorphism at position -786 in the promoter region and single nucleotide polymorphisms in exon 7 (298Glu/Asp or 5557G/T) of the eNOS gene.

Statistical analyses

Strength of association between EN and eNOS alleles or genotypes 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 $p < 0.05$. Estimated haplotype frequencies and testing for linkage disequilibrium (LD) between pairs of polymorphisms in cases and controls were calculated using the EHPLUS program, which provides log likelihood, chi-square and the number of degrees of freedom.

Results

No significant differences in the allele and genotype distribution between patients with EN and controls were observed (Table I). Also, no significant differences were observed when patients with sarcoidosis were compared with the remaining EN patients or controls. (Table I).

Pairwise LD and estimate haplotype frequencies were determined in patients with EN and controls. In the control group a marginally significant LD (Chi square 7.5; $p = 0.05$) was found between eNOS promoter (-786) and exon 7 polymorphism. However, this was not statistically significant in the patient's group. Likewise, no significant difference in pairwise LD was observed in patients with EN compared with controls using log likelihood. Moreover, no significant differences in haplotype frequencies were seen in patients with EN compared to controls. Similarly, haplotype frequencies for the combination of the three eNOS polymorphisms did not yield significant LD in the patient's group (data not shown).

Discussion

This study constitutes the first attempt to determine the potential implication of three functional polymorphisms in eNOS in the susceptibility to EN. The clinical heterogeneity of conditions presenting with EN may explain the negative association observed between eNOS polymorphisms in this series of patients with biopsy-proven secondary EN. Due to this, the main importance of this study is the lack of role of eNOS polymorphisms in patients with primary (idiopathic) EN from Northwestern Spain.

Polymorphisms in eNOS have been associated with the development of vascular and autoimmune diseases. In this regard, association between eNOS

polymorphisms and coronary atherosclerosis and ischemic cerebrovascular disease has been previously reported (16, 17). Also, the polymorphism of the exon 7 (Glu/Asp 298) was associated with susceptibility to Behçet's disease and scleroderma (7, 18). In keeping with Salvarani *et al*. (5), association between biopsy-proven giant cell arteritis and eNOS polymorphisms was also observed in the Lugo region of Northwestern Spain (6).

The lack of association of EN with eNOS polymorphisms do not exclude a potential association of this condition with iNOS polymorphisms. With respect to this, association with iNOS but not with eNOS polymorphisms has been observed in individuals with Henoch-Schönlein purpura (15, 19) or rheumatoid arthritis (20) from the Lugo region of Northwestern Spain. Due to this, studies aimed to determine the potential association of iNOS polymorphisms with biopsy-proven EN are required to further elucidate the role of NO polymorphisms in the pathogenesis of EN.

References

1. GONZALEZ-GAY MA, GARCIA-PORRUA C, PUJOL RM, SALVARANI C: Erythema nodosum: a clinical approach. *Clin Exp Rheumatol* 2001; 19:365-8.
2. ATZENI F, CARRABBA M, DAVIN JC *et al*.: Skin manifestations in vasculitis and erythema nodosum. *Clin Exp Rheumatol* 2006; 24 (Suppl. 40): S60-6.
3. CLANCY RM, AMIN AR, ABRAMSON SB: The role of nitric oxide in inflammation and immunity. *Arthritis Rheum* 1998; 41: 1141-51.
4. WEINBERG JB: Nitric oxide production and nitric oxide synthase type 2 expression by human mononuclear phagocytes: a review. *Mol Med* 1998; 4: 557-91.
5. SALVARANI C, CASALI B, NICOLI D *et al*.: Endothelial nitric oxide synthase gene polymorphisms in giant cell arteritis. *Arthritis Rheum* 2003; 48: 3219-23.
6. AMOLI MM, GARCIA-PORRUA C, LLORCA J, OLLIER WE, GONZALEZ-GAY MA: Endothelial nitric oxide synthase haplotype associations in biopsy-proven giant cell arteritis. *J Rheumatol* 2003; 30: 2019-22.
7. SALVARANI C, BOIARDI L, CASALI B *et al*.: Endothelial nitric oxide synthase gene polymorphisms in Behçet's disease. *J Rheumatol* 2002; 29: 535-40.
8. AMOLI MM, DONN RA, THOMSON W *et al*.: Macrophage migration inhibitory factor gene polymorphism is associated with sarcoidosis in biopsy-proven erythema nodosum: *J Rheumatol* 2002; 29: 1671-3.
9. AMOLI MM, LLORCA J, GOMEZ-GIGIREY A *et al.:* E-selectin polymorphism in erythema nodosum secondary to sarcoidosis. *Clin Exp Rheumatol* 2004; 22: 230-2.
10. GARCIA-PORRUA C, GONZALEZ-GAY MA, VAZQUEZ-CARUNCHO M *et al*.: Erythema nodosum: etiologic and predictive factors in a defined population. *Arthritis Rheum* 2000; 43: 584-92.
11. AMOLI MM, THOMSON W, HAJEER AH *et al*.: HLA-DRB1 associations in biopsy-proven erythema nodosum. *J Rheumatol* 2001; 28: 2660-2.
12. GONZALEZ-GAY MA, GARCIA-PORRUA C, HAJEER AH: The HLA-DRB1 influence in the susceptibility and severity to rheumatoid arthritis. *Semin Arthritis Rheum* 2002; 31: 355-60.
13. GONZALEZ-GAY MA, ALONSO MD, AGUERO JJ, BAL M, FERNANDEZ-CAMBLOR B, SANCHEZ-ANDRADE A: Temporal arteritis in a northwestern area of Spain: study of 57 biopsy proven patients. *J Rheumatol* 1992; 19: 277-80.
14. GONZALEZ-GAY MA, GARCIA-PORRUA C, GUERRERO J, RODRIGUEZ-LEDO P, LLORCA J: The epidemiology of the primary systemic vasculitides in northwest Spain: implications of the Chapel Hill Consensus Conference definitions. *Arthritis Rheum* 2003; 49: 388-93.
15. AMOLI MM, GARCIA-PORRUA C, CALVINO MC, OLLIER WE, GONZALEZ-GAY MA: Lack of association between endothelial nitric oxide synthase polymorphisms and Henoch-Schonlein purpura. *J Rheumatol* 2004; 31: 299-301.
16. NAKAYAMA M, YASUE H, YOSHIMURA M *et al*.: T-786 C mutation in the 5'-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. *Circulation* 1999; 99: 2864-70.
17. MARKUS HS, RUIGROK Y, ALI N, POWELL JF: Endothelial nitric oxide synthase exon 7 polymorphism, ischemic cerebrovascular disease, and carotid atheroma. *Stroke* 1998; 29: 1908-11.
18. FATINI C, GENSINI F, STICCHI E *et al*.: High prevalence of polymorphisms of angiotensin-converting enzyme (I/D) and endothelial nitric oxide synthase (Glu298Asp) in patients with systemic sclerosis. *Am J Med* 2002; 112: 540-4.
19. MARTIN J, PACO L, RUIZ MP, LOPEZ-NEVOT MA *et al*.: Inducible nitric oxide synthase polymorphism is associated with susceptibility to Henoch-Schönlein purpura in northwestern Spain. *J Rheumatol* 2005; 32: 1081-5.
20. GONZALEZ-GAY MA, LLORCA J, SANCHEZ E *et al*.: Inducible but not endothelial nitric oxide synthase polymorphism is associated with susceptibility to rheumatoid arthritis in Northwest Spain. *Rheumatology (Oxford)* 2004; 43: 1182-5.