

Association of the A561C E-Selectin Polymorphism with Systemic Lupus Erythematosus in 2 Independent Populations

MASOUD EL-MAGADMI, ALIYA ALANSARI, LEE-SUAN TEH, JOSE ORDI, AHMET GÜL, MURAT INANC, IAN BRUCE, and ALI HAJEER

ABSTRACT. *Objective.* E-selectin is expressed on cytokine stimulated endothelial cells and plays an important role in leukocyte-endothelium interactions and inflammatory cell recruitment. The gene for E-selectin is located at chromosome 1q 23–25 within the linkage area for systemic lupus erythematosus (SLE). The best characterized polymorphism in E-selectin molecule is A561C, which codes for Ser128Arg. We studied the prevalence of the A561C E-selectin gene polymorphism in patients with SLE and controls from 3 different ethnic populations.

Methods. Three cohorts of patients with SLE (1987 American College of Rheumatology criteria) and matching population controls were studied. These consisted of Caucasians of British Isles descent, Caucasians of Spanish origin, and Caucasians of Turkish origin. We used polymerase chain reaction and restriction fragment length polymorphism to genotype patients and controls.

Results. The numbers of patients and controls in each group were: UK (113 and 148), Spanish (145 and 179), and Turkish (93 and 96), respectively. The C allele occurred more frequently in UK and Spanish patients (OR 1.76, 95% CI 1.03–3.0, $p = 0.037$; and OR 1.84, 95% CI 1.1–3.09, $p = 0.019$), but not in Turkish patients (OR 1.03, 95% CI 0.55–1.97, $p = 0.91$).

Conclusion. In 2 of 3 populations studied, the E-selectin C allele was significantly more common in SLE than in controls. E-selectin may be a susceptibility gene to SLE in these populations. Its role in disease expression and longterm outcomes such as accelerated atherosclerosis requires further study. (J Rheumatol 2001;28:2650–2)

Key Indexing Terms:

SYSTEMIC LUPUS ERYTHEMATOSUS
E-SELECTIN

GENETICS

ASSOCIATION STUDIES
ETHNICITY

The etiology of systemic lupus erythematosus (SLE) is unknown; however, twin and family studies suggest that both genetic and environmental factors influence susceptibility. Linkage studies in murine models and in human SLE have located a susceptibility region on the syntenic region of chromosome 1 (1q 21–42)¹. This region contains several relevant genes including several immunomodulatory,

complement, autoantigens, and apoptosis related genes. The genes for the selectin group of cellular adhesion molecules are located in this region (1q 22–25). There are 3 types of selectins, E, L, and P-selectin, expressed on endothelial cells, leukocytes and platelets, and endothelium, respectively. They are structurally similar transmembrane glycoproteins consisting of an N-terminal-like domain, an epidermal growth factor (EGF)-like domain connected with variable repeats of amino acid units to a membrane and cytoplasmic domain, and they bind to specific carbohydrate molecules on the leukocyte surface². E-selectin is expressed on most of the leukocyte subset, and its expression is tightly regulated by inflammatory cytokines, mainly interleukin 1 and tumor necrosis factor- α . On cytokine activation, E-selectin appears within 1 hour, reaches maximum at 6 hours, and then gradually returns to basal level³. Initial rolling of leukocytes on E-selectin activates $\beta 2$ integrin, which binds intercellular adhesion molecule 1, leading to cell arrest⁴. *In vivo* E-selectin expression correlates with early polymorphonuclear cells and later with lymphocyte infiltration; blocking of E-selectin with monoclonal antibodies results in 60% decrease in infiltration of both cell types⁵.

Three nucleotide polymorphisms have been described in

From the ARC Epidemiology Research Unit, School of Epidemiology and Health Sciences, The University of Manchester, Manchester, United Kingdom.

Supported by the UK Arthritis Research Campaign.

M. El-Magadmi, MB, ChB, Research Fellow; A. Alansari, MSc, Research Fellow, ARC Epidemiology Research Unit; L-S Teh, PhD, FRCP, Consultant Rheumatologist, Blackburn Royal Infirmary, Blackburn, UK; J. Ordi, MD, Hospital General Vall d'Hebron, Barcelona, Spain; A. Gül, MD; M. Inanc, Division of Rheumatology, Istanbul Faculty of Medicine, University of Istanbul, Turkey; I.N. Bruce, MD, MRCP, Consultant Rheumatologist and Honorary Clinical Lecturer, Central Manchester Healthcare NHS Trust and University of Manchester; A.H. Hajeer, PhD, MRCPath, Lecturer in Immunogenetics, ARC Epidemiology Research Unit.

Address reprint requests to Dr. A. Hajeer, ARC Epidemiology Research Unit, School of Epidemiology and Health Sciences, Stopford Building, The University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom. E-mail: Ali.Hajeer@man.ac.uk

Submitted January 17, 2001; revision accepted June 25, 2001.

E-selectin gene: G98T is in the 5' untranslated region; C1839T and A561C are associated with amino acid exchange; C1839T results in exchange of leucine to phenylalanine (L554F) in the membrane domain. Mutation from adenine to cytosine at position 561 (A561C) results in amino acid change from serine to arginine at position 128 (S128R) in the EGF-like domain near the lectin binding site (C-allele). G98T mutation is in strong linkage disequilibrium with the A561C in EGF domain, while L55F has a low mutation rate⁶. E-selectin may therefore represent a candidate gene in SLE. We examined the frequency of the E-selectin A561C polymorphism in 3 different SLE populations.

MATERIALS AND METHODS

Three Caucasian SLE populations were studied and compared to ethnic and geographically matched controls — Caucasians of British Isles descent, Caucasians of Spanish origin, and Caucasians of Turkish origin. All cases fulfilled the 1987 American College of Rheumatology (ACR) revised criteria for SLE. We designed primers to amplify the 249 base-pair (bp) genomic segment of the E-selectin gene containing the polymorphic site. The primer sequences used were forward (5'-GTC TCA GCT CAC GAT CAC CA-3') and reverse (5'-CCG TAG CTG CCT GTA CCA AT-3'). Polymerase chain reaction (PCR) was carried out in a total volume of 25 μ l and was optimized to an initial cycle of 2 min denaturation at 94°C followed by 35 cycles of denaturation for 1 min at 94°C. Annealing was at 55°C for 1 min, and synthesis for 1 min at 72°C. This was followed by extension at 72°C for 5 min. Seven microliters of the PCR product were digested with 4 units of *Pst* I restriction enzyme overnight at 37°C; digestion products were separated on 3% agarose gel electrophoresis. The A561C polymorphism inhibits the restriction site for the *Pst* I enzyme. Therefore in the homozygous genotype A/A the PCR product is seen as 2 fragments of 219 and 30 bp. The homozygous mutant genotype C/C product appears as one band of 249 bp and the heterozygous genotype A/C produces 3 fragments, 249, 219, and 30 bp.

Statistical analysis was carried out using Stata V.6 program. The chi-square test was used to compare the proportions of the 3 genotypes in the cases and controls; odds ratios (OR) with 95% confidence intervals (CI) were calculated for the allele frequencies.

RESULTS

We studied 113 patients and 148 controls from the UK, 113 patients and 145 controls from Spain, and 93 patients and 96 controls from Turkey. The frequency of the C allele was

significantly higher in UK and Spanish Caucasian patients compared to their respective controls; this was not seen in those of Turkish origin (Table 1). OR (95% CI) for the presence of the C-allele in SLE in UK and Spanish patients was 1.67 (1.03–3.0) and 1.84 (1.1–3.1), respectively. We found no significant association between this allele and specific clinical features, i.e., nephritis, central nervous system involvement, or production of antibodies to double stranded DNA. Precise information regarding Systemic Lupus International Collaborating Clinics/ACR damage index and atherosclerotic outcomes was not available.

DISCUSSION

We found an association between SLE and the E-selectin A561C polymorphism in 2 independent populations. The strength of the association, with OR of 1.6–1.8, is also consistent with this polymorphism being one of several genetic markers that are likely to contribute to disease susceptibility in SLE in a particular population. The lack of association in the Turkish patients can be due to sample size effect or could support our previous observation that genetic associations in SLE vary with ethnicity⁷.

The C allele has been the subject of much debate and controversy. The exact role of this polymorphism requires clarification. The Ser128Arg mutation has been found to alter the binding specificity to carbohydrate molecules on the leukocytes and increases leukocyte binding by 2–3-fold compared to the wild-type gene⁸. However, others have shown that the Ser128Arg mutation diminished the release of soluble E-selectin and significantly reduced binding⁹. These studies have been performed on static models. A recent study comparing the S128R E-selectin polymorphism to wild-type E-selectin in myeloid cell lines under flow conditions showed increased tethering and adhesion associated with the S128R polymorphism¹⁰. Enhanced recruitment of inflammatory cells may therefore provide a pathogenic explanation for the association we observed and requires further study.

Recruitment of inflammatory cells to the subendothelial space is also an early process in the pathogenesis of athero-

Table 1. Genotype and allele frequencies in 3 distinct ethnic SLE groups and matched controls.

Group	Genotype			Alleles		OR (95% CI)	p
	AA n (%)	AC n (%)	CC n (%)	A n (%)	C n (%)		
UK							
Cases	85 (75.2)	22 (19.5)	6 (5.3)	192 (85.0)	34 (15)	1.67 (1.03–3.0)	0.037
Controls	122 (82.4)	25 (16.9)	1 (0.7)	269 (90.9)	27 (9.1)		
Spanish							
Cases	110 (76)	32 (22)	3 (0.2)	252 (86.9)	38 (13.1)	1.84 (1.10–3.1)	0.019
Controls	153 (85.4)	25 (14)	1 (0.6)	331 (93.0)	27 (7.0)		
Turkish							
Cases	74 (79.6)	16 (17.2)	3 (3.2)	164 (88.2)	22 (11.8)	1.03 (0.55–1.97)	0.91
Controls	75 (78.1)	20 (20.8)	1 (1.0)	170 (89.6)	22 (10.4)		

sclerosis. Several studies have found an association between the S128A E-selectin polymorphism and premature coronary artery disease in the general population, especially in subjects < 40 years of age^{11,12}. Given that accelerated atherosclerosis is a recognized late feature of SLE¹³, this may also provide insight into the common immune/inflammatory origins of both vascular pathologies.

This study has several limitations. First, our groups contained relatively small numbers of patients; it is therefore important that this finding be reproduced in other SLE cohorts and explored in other ethnic subgroups. Second, while all patients fulfilled the classification criteria for SLE, our data related only to the clinical features included in the ACR criteria and a profile of autoantibodies ever positive in these patients. Our study is limited in its ability to detect any associations of this polymorphism in distinct disease subgroups. In addition, data on coronary artery disease were not available. A larger and more detailed study is now under way to explore these issues.

We found an association of the A561C E-selectin polymorphism with SLE in 2 independent populations. This polymorphism may be directly implicated in the disease susceptibility or it may be in linkage disequilibrium with another polymorphism in the vicinity of the E-selectin gene. The pivotal role of E-selectin in leukocyte-endothelial cell interactions may increase susceptibility to vascular inflammation in SLE. Further studies are now needed to assess if it also influences the clinical phenotype and particularly whether it may increase susceptibility to accelerated atherosclerosis seen in SLE.

REFERENCES

1. Tsao BP, Cantor RM, Kalunian KC, et al. Evidence for linkage of a candidate chromosome 1 region to human systemic lupus erythematosus. *J Clin Invest* 1997;99:725-31.
2. Bevilacqua MP. Endothelial-leukocyte adhesion molecules. *Annu Rev Immunol* 1993;11:767-804.
3. Wellicome SM, Thornhill MH, Thomas DS, Lanchbury JS, Panayi GS, Haskard DO. A monoclonal antibody that detects a novel antigen on endothelial cells that is induced by tumour necrosis factor, IL-1 or lipopolysaccharide. *J Immunol* 1990;144:2558-65.
4. Simon SI, Hu Y, Vestweber D, Smith CW. Neutrophil tethering on E-selectin activates beta 2 integrin binding to ICAM-1 through a mitogen-activated protein kinase signal transduction pathway. *J Immunol* 2000;164:4348-58.
5. Binns RM, Licence ST, Harrison AA, Keelan ET, Robinson MK, Haskard OD. In vivo E-selectin up regulation correlates early with infiltration of PMN, later with PBL entry: Mabs block both. *Am J Physiol Heart Circ Physiol* 1996;39:H183-H193.
6. Wenzel K, Ernst M, Rohde K, Baumann G, Speer A. DNA polymorphism in the adhesion molecule genes — a new risk for early atherosclerosis. *Hum Genet* 1996;97:15-20.
7. Al-Ansary AS, Ollier WER, Villarreal J, Ordi J, Tech L-S, Hajeer AH. Tumour necrosis factor receptor II exon 6 polymorphism in systemic lupus erythematosus. *Tissue Antigens* 2000;55:97-9.
8. Revelle BM, Scott D, Beck PJ. Single amino acid residues in the E- and P-selectins epidermal growth factor domains can determine carbohydrate binding specificity. *J Biol Chem* 1996;271:16160-70.
9. Wenzel K, Stahn R, Speer A, et al. Functional characterization of atherosclerosis-associated Ser128Arg and Leu554Phe E-selectin mutations. *Biol Chem* 1999;380:661-7.
10. Rao RM, Clarke JL, Robinson MK, Landis C, Haskard DO. The E-selectin S128A polymorphism enhances tethering and firm adhesion of myeloid cells lines under flow [abstract]. *Arthritis Rheum* 2000;43 Suppl:S89.
11. Wenzel K, Felix S, Kleber FX, et al. E-selectin polymorphism and atherosclerosis: an association study. *Hum Mol Genet* 1994; 3:1935-7.
12. Ye SQ, Usher D, Virgil D, Zhang LQ, Yochim SE, Gupta R. A pstI polymorphism detects the mutation of serine to arginine in CD 62E gene — a risk factor for coronary artery disease. *J Biomed Sci* 1999;6:18-21.