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E-selectin and L-selectin polymorphisms in patients with periodontitis

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Background amd Objective: Periodontitis is a multifactorial disease in which environmental and genetic determinant factors contribute to individual subject's susceptibility. A DNA polymorphism in the regulating region of adhesion molecule genes is suggested to modulate the molecule's physiological effects. The aim of this study was to investigate the genetic association between the E-selectin Ser128Arg and L-selectin Phe206Leu polymorphisms and periodontitis.

Material and Methods: DNA was isolated from the whole blood of 88 patients with periodontitis and 139 healthy individuals. All samples were genotyped for the E-selectin Ser128Arg and L-selectin Phe206Leu polymorphisms using the polymerase chain reaction with sequence specific primers.

Results: Our findings revealed a significant difference in the Ser128Arg polymorphism of E-selectin, but not in the L-selectin polymorphism, between periodontal patients and controls. The 128Arg allele was present more frequently in patients than in healthy individuals (31.25% vs. 12.2%, $p < 0.0001$). In addition, there was an association between the presence of the 128Arg allele and periodontitis (odds ratio 2.9; 95% confidence interval: 1.75–4.4, $p < 0.0001$). No significant association was found between the polymorphisms tested and the subgroups of periodontal disease (i.e. chronic periodontitis and aggressive periodontitis).

Conclusion: The findings of this study showed that the Ser128Arg polymorphism of E-selectin might contribute to the susceptibility of Iranian individuals to periodontitis.

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The periodontium, which anchors the teeth to the jaws, consists of the gingiva, periodontal ligament, cementum and alveolar bone (1). It is normally in a balanced state with the periodontal microbiota in the dental plaque. Human periodontal diseases (i.e. gingivitis and periodontitis) result from heterogenous etiologies, including changes to the complex biofilm in the subgingivial microenvironment, social and behavioral modulations, and genetic or epigenetic traits of the host's immune and inflammatory responses (2). In fact, periodontitis is a chronic inflammatory disease that is characterized by destructive inflammatory processes affecting the supporting structures of the teeth, causing resorption of alveolar bone and formation of periodontal pockets (3). The risk for development and/or progression of periodontitis is thought to be determined in part by the host's genotype (4).

Leukocyte extravasation is part of the inflammatory reaction, which is an important process in self-defense mechanisms at sites of inflammation as well as in lymphocyte homing. It is also important as a process responsible for the pathogenesis of inflammatory disorders (5). The migration of leukocytes into sites of inflammation is mediated by adhesion molecules and consists of leukocyte rolling on the endothelium of postcapillary venules, followed by their firm adhesion and migration into prevascular tissues (6). The early adhesion events that facilitate leukocyte rolling predominantly involve selectin-carbohydrate interactions (7). L-selectin is a member of the selectin

family, which is constitutively expressed on leukocytes such as peripheral lymphocytes, monocytes and neutrophils. E-selectin, another member of the selectin family, is exclusively expressed on activated endothelium stimulated by inflammatory cytokines such as interlukin-1, tumor necrosis factorα and lipopolysaccharides (6,7).

While microbial and other environmental factors are believed to initiate and modulate periodontal progression, the fact remains that not everyone appears to be equally susceptible to periodontal diseases (8). There exists strong supporting evidence that genes play a role in the predisposition to and progression of periodontal disease (4,9,10). Relationships between genes regulating immune responses and periodontitis seem to be intuitively reasonable given the current dogma regarding protective and destructive immune responses in the pathogenesis of periodontitis and attachment loss (10).

Genetic polymorphisms have been associated with different clinical forms of periodontitis. Several polymorphisms have been described within the selectin gene cluster (11,12). The two polymorphisms investigated in the present study are E-selectin (A561C) and L-selectin (T668C). In the E-selectin (A561C) polymorphism, an adenine to cytosine substitution in the coding region of the E-selectin gene results in an amino acid exchange from serine to arginine at codon 128 (Ser128Arg). This substitution takes place in the epidermal growth factor-like domain of this molecule, which confers an alteration in selectin ligand-binding specificity, leading to a gain of function under flow conditions, possibly amplifying the number of leukocytes that roll and subsequently arrest on the endothelium (13). It also extends the range of lymphocytes recruited by E-selectin (13,14). In the L-selectin (T668C) polymorphism, a phenylalanine to leucine exchange (Phe206Leu) in the epidermal growth factor-like domain of L-selectin may alter its ligand interactions (15).

The polymorphisms investigated in the present study have been previously associated with diseases/conditions such as coronary artery disease (16,17), atherosclerosis (18,19), high blood pressure (20), systemic lupus erythromatosus (21) and brucellosis (22). As no study has yet been undertaken on the possible association between the E-selectin Ser128Arg (A561C) and L-selectin Phe206Leu (T668C) polymorphisms and periodontal disease, we evaluated their contribution in the susceptibility to periodontitis.

Material and methods

Study population

A sample size calculation with alpha 0.05 and beta 0.80 was performed to estimate the patient number needed to detect relevant differences in periodontitis between different E-selectin and L-selectin genotypes. Therefore, a total of 227 subjects who had never smoked were recruited for the study. Eighty-eight patients (55 women and 33 men; age range: 15–60 years), consisting of 41 patients with aggressive periodontitis and 47 patients with chronic periodontitis were enrolled. Periodontitis was diagnosed based on the patient's medical and dental histories, and on clinical signs and parameters, such as probing depth, clinical attachment loss, bleeding on probing index, the presence of plaque and sequential radiographic findings (16). No patient had a history of current manifestation of systemic diseases. Patients with severe medical disorders (such as diabetes mellitus, immunological disorders, hepatitis, human immunodeficiency virus infection and cardiovascular involvement), those who used orthodontic appliances, those with a need for premedication for dental treatment, with chronic usage of anti-inflammatory drugs and who were presently experiencing acute necrotizing ulcerative gingivitis, and pregnant or lactating woman, were excluded from the study.

The diagnostic criteria of chronic periodontitis were as follows: (i) the amount of periodontal destruction was consistent with the presence of local factors such as plaque and calculus; and (ii) at least two sites had a probing depth of ≥ 5 mm and clinical attachment loss of ≥ 1 mm in every quadrant.

The clinical criteria of aggressive periodontitis were: interproximal attachment loss, affecting at least three permanent teeth other than central incisors or first molars; a probing depth of ≥ 5 mm; and clinical attachment loss of ≥ 3 mm. On the regular medical records, all patients at the diagnosis of disease were younger than 35 years. In addition, 139 healthy volunteers (73 women and 66 men; age-range 18–53 years) as a control group, matched with no evidence of periodontitis, were included in the study. The healthy control group included subjects who were selected from employees and professionals at the dental school with similar socio-economic and ethnic backgrounds to the patients. They originated from the same area in the southwestern part of Iran. To evaluate the socio-economic characteristics of individuals, such as education, employment status, family monthly income and type of residence, we used a questionnaire for all individuals enrolled in this study. The monthly income was recorded in Iranian currency (rials), which was converted into US dollars. None of the control subjects had a history of periodontitis or tooth loss as a result of pathogenic tooth mobility. To minimize random and systematic errors, all measurements were performed by a single examiner, who was an experienced periodontologist (HB). All subjects signed an informed consent form before enrolment into the study.

Genotyping

Ten millilitres of venous blood was collected from each subject into tubes containing 50 mM/L of EDTA, and genomic DNA was isolated from anticoagulated peripheral blood buffy coat using Miller's salting-out method (23). Then, the genomic DNA was stored at -80°C until required for genotyping. The genotyping was performed using the polymerase chain reaction (PCR) sequence-specific primers method (24). Internal control primers were included to control for false-negative reactions. The control primers at a concentration

of 0.2 μM (5-GCC TTC CCA ACC ATT CCC TT-3′ and 5-TCA CGG ATT TCT GTT GTG TTT C-3′) were used to amplify a 796-bp segment of the *HLA-DR4* gene.

The E-selectin polymorphism at position A561C was identified using the sequence-specific forward primers: 5′-CTG TAC CAA TAC ATC CTG CA-3′ and 5′-CTG TAC CAA TAC ATC CTG CC-3′ in combination with the consensus reverse primer 5′-TCT GAC TTC ATA GTC TCA GCT-3′. In addition, the L-selectin T668C polymorphism was revealed by the sequence-specific forward primers: 5′-ATG GGC CCC AGT GTC AGT-3′ and 5′-ATG GGC CCC AGT GTC AGC-3′ in combination with the common reverse primer 5′-CAA GCT CAT TAG ATC GTG AGC-3′.

Amplification was carried out using a DNA Technology MTC 410 (DNA Technology, Moscow, Russia) in a total volume of 15 μL that contained 100 ng of genomic DNA, 1 mM each allele-specific primer pair, 200 μM dNTP, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.5 IU Taq DNA polymerase.

The reaction was carried out as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of amplification at 96°C for 20 s and annealing at 64°C for 50 s, with extension for 40 s at 72°C and a final extension for 2 min at 72°C. The amplified PCR products were analyzed by electrophoresis on a 2% agarose gel followed by staining in ethidium bromide (0.5 μg/mL) and visualization under ultraviolet light. The genotyping was performed blindly to clinical diagnosis. In other words, the agarose-gel electrophoresis results were reported by an investigator unaware of the origin of the samples. Independent quality control analysis was performed by a laboratory technician; for the Ser128Arg variant this consisted of PCR and genotyping of a random selection of 20% of the patients and controls to test for any differences compared with the initial genotype data. Repetition of the genotyping on random samples revealed no differences compared with the initial data in all samples tested.

Data analysis

Allelic and genotypic frequencies were obtained by direct counting. Contingency tables were used with chi-square tests to compare observed genotype frequencies with those expected under Hardy–Weinberg equilibrium. Chi-square and Fisher's exact tests were used to test for differences in genotype and allele distribution between the groups. Unconditional logistic regression models were applied to estimate odds ratio and 95% confidence intervals for the genetic risk factor in the total study population and in the subgroups of periodontitis. A p-value of < 0.05 was considered statistically significant.

Results

The characteristics of the patient and control groups are shown in Table 1. The mean age was 44.1 ± 5.3 and 35.7 ± 7.6 years in the patients and healthy individuals, respectively.

No socio-economic status differences between the patients with periodontitis and the control individuals were observed. There was no significant difference in the illiteracy rate between the patients (6.41%) and the controls (4.87%). Among the controls, 73% voluntarily provided their monthly income compared with 58% of patients with periodontitis. The monthly income of controls was not significantly different from that of patients ($p < 0.12$). There were also no significant differences in the employment status and type of residency between control and patient groups.

DNA samples from 88 patients with periodontal disease and from 139 healthy individuals were analyzed for the presence of the Ser128Arg polymorphism of E-selectin and for the presence of the Phe206Leu polymorphism of L-selectin. The frequencies of these two selectin genotypes in control individuals were found to be in accordance with those expected by Hardy–Weinberg equilibrium. Testing for deviation from Hardy–Weinberg equilibrium was performed according to the program FINETTI (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl/). An increased frequency of the E-selectin mutant C allele (128Arg) was observed in the patients compared with the controls (31.25% vs. 12.2%, $p < 0.0001$) (Table 2). Logistic regression analysis in the study groups was utilized to investigate the independent role of the genotype frequency in susceptibility to periodontitis and to adjust the potential confounding effect of the age difference that was observed between the patients and the controls. The logistic regression analysis results indicated that the C allele in patients was associated with a higher risk for the disease compared with controls (odds ratio 2.9; 95% confidence interval: 1.75–4.43, $p < 0.0001$). Indeed, these results derive from a significantly increased number of individuals with the Arg/Arg genotype in a population of patients with periodontitis compared with healthy subjects (15.9% vs. 3.6%; $p < 0.0001$).

In addition, as shown in Table 3, the allele and genotype frequencies of the Phe206Leu polymorphism of the L-selectin gene were not significantly different between periodontitis

Table 1. Clinical and demographic data of patients with periodontal disease and of healthy controls

Group	*n*	Men (%)	Women (%)	Age (years)	Probing depth (mm)	Clinical attachment loss (mm)	Bleeding on probing index (%)	Plaque control record (%)
Aggressive periodontitis	41	17 (41.5)	24 (58.5)	37.3 ± 7.1	6.3 ± 1.01	5.07 ± 0.97	88.9 ± 6.3	58.2 ± 14.6
Chronic periodontitis	47	21 (44.7)	26 (55.3)	49 ± 6.7	5.1 ± 1.37	3.96 ± 1.2	82.96 ± 9.6	88.6 ± 6.4
Periodontitis (combined)	88	38 (43.2)	50 (56.8)	43.5 ± 9	5.7 ± 1.34	4.53 ± 1.2	86 ± 8.5	73.1 ± 19.
Healthy controls	139	66 (47.5)	73 (52.5)	35.8 ± 8.6	1.7 ± 0.2	1.8 ± 0.3	6.3 ± 0.75	35.2 ± 14.2

Table 2. Allele and genotype frequencies of the E-selectin (Ser128Arg) polymorphism in patients with periodontitis and in controls

E-selectin (Ser128Arg) polymorphism	Periodontitis $n = 88$	Controls $n = 139$	p-value
Allele, *n* (%)			
Ser	121 (68.75)	216 (87.8)	< 0.0001
Arg	55 (31.25)	30 (12.2)	
Genotype, *n* (%)			
Ser/Ser	47 (53.4)	105 (75.5)	< 0.0001
Ser/Arg	27 (30.7)	29 (20.9)	
Arg/Arg	14 (15.9)	5 (3.6)	

The values represent the numbers (percentages) of patients or controls who were positive for each allele or genotype. The p values were calculated using the chi-squared test from 3×2 or 2×2 contingency tables for genotypes and alleles, respectively.

Table 3. Allele and genotype frequencies of the L-selectin (Phe206Leu) polymorphism in patients with periodontitis and in controls

L-selectin (Phe206Leu) polymorphism	Periodontitis $n = 88$	Controls $n = 139$	p-value
Allele, *n* (%)			
Phe	71 (40.34)	94 (33.81)	0.16
Leu	105 (59.66)	184 (66.19)	
Genotype, *n* (%)			
Phe/Phe	14 (15.9)	19 (13.7)	0.27
Phe/Leu	43 (48.9)	56 (40.3)	
Leu/Leu	31 (35.2)	64 (46)	

The values represent the numbers (percentages) of patients or controls who were positive for each allele or genotype. The p values were calculated using the chi-squared test from 3×2 or 2×2 contingency tables for genotypes and alleles, respectively.

(aggressive or chronic combined) subjects and controls ($p > 0.05$). Stratification of periodontitis patients according to the disease phenotype (i.e. aggressive periodontitis or chronic periodotitis) showed no significant differences in the frequency of either E-selectin Ser128Arg ($p = 0.8$) or L-selectin Phe206Leu ($p = 0.135$) single nucleotide polymorphisms between patients with aggressive periodontitis and those with chronic periodontitis (Table 4).

When stratifying patients by gender or socio-economic status, no statistically significant differences were observed for either single nucleotide polymorphisms (data not shown).

Discussion

Periodontitis is an inflammatory disorder that is characterized by the destruction of connective tissue and alveolar bone. The correlation of known polymorphisms in components of the human immune system with phenotypes for certain patient groups currently appear to provide the most promising application of genetic determinants of periodontitis. According to our findings, the frequencies of the 128Arg allele and the genotype of the E-selectin single nucleotide polymorphism were higher in patients with periodontitis than in healthy individuals, suggesting that the E-selectin polymorphism might influence the susceptibility of individuals to periodontitis. This single nucleotide polymorphism in the E-selectin gene has been demonstrated to exert a profound effect on ligand recognition and binding, as cell transfectants expressing the mutant allele of this polymorphism better support interaction with leukocytes under flow conditions (14). This could theoretically amplify the number of lymphocytes and other leukocytes interacting with endothelial cells expressing the mutant form of the molecule during periodontal inflammatory responses to bacterial plaque.

To date, there have been no reports of association analysis between the single nucleotide polymorphisms of selectin and susceptibility to periodontitis. However, we have recently reported an association between the E-selectin Ser128Arg polymorphism and a subgroup of patients with brucellosis who had a short onset of disease (25). E-selectin has an N-terminal C-type lectin domain that is thought to process the carbohydrate-binding site that binds to E-selectin ligands (26). Substitution of an uncharged serine with a positively charged arginine at residue 128 of the epidermal growth factor domain may influence E-selectin function, either directly or by inducing a conformation change in the lectin domain of the molecule. The relationship between the E-selectin Ser128Arg polymorphism and periodontitis may reflect an amplified inflammatory

Table 4. Allele and genotype frequencies for Ser128Arg of the E-selectin polymorphism and for Phe206Leu of the L-selectin polymorphism in aggressive and chronic periodontitis

	E-selectin Ser128Arg					L-selectin Phe206Leu				
	Ser/Ser (%)	Ser/Arg (%)	Arg/Arg (%)	Ser (%)	Arg (%)	Phe/Phe (%)	Phe/Leu (%)	Leu/Leu (%)	Phe (%)	Leu (%)
Aggressive periodontitis	21 (51.2)	14 (34.1)	6 (14.6)	56 (68.3)	26 (31.7)	5 (12.2)	21 (51.2)	15 (36.6)	31 (37.8)	51 (62.2)
Chronic periodontitis	26 (55.3)	13 (27.7)	8 (17)	65 (69.1)	29 (30.9)	14 (29.8)	19 (40.4)	14 (29.8)	47 (50)	47 (50)

No statistical differences were observed for any comparison (chi-squared test, $p > 0.05$).

response resulting from the action of altered selectin molecules containing the serine to arginine mutation. The substitution of arginine for serine has been shown to decrease the binding specificity dramatically, while increasing the affinity for additional ligands, resulting in an increase in the cellular adhesion of two- to threefold (13). The E-selectin 128Arg allele may thus increase leukocyte adherence to activated endothelium in areas susceptible to the formation of bacterial plaque, thereby contributing to the progression of periodontitis. Functional studies have demonstrated a reduced strength of adhesion associated with the Arg allele (27).

Because L-selectin also has a role in attracting leukocytes to inflammatory sites (28), we analyzed the L-selectin Phe206Leu polymorphism in patients with periodontitis. Although recent studies have addressed the significant association between the Phe206Leu polymorphism of L-selectin with certain disorders such as immunoglobulin A nephropathy (29) and brucellosis (22), we were not able to detect any statistically significant association of this polymorphism with periodontitis. This finding indicates that different genes appear to be related to host susceptibility for periodontitis.

Although the principal ligand contact point of the selectins lies within the lectin domain (30), the substitution of a phenylalanine with a leucine at residue 206 may influence L-selectin binding function. It may be speculated that the pathogenic mechanisms underlying the development of some complicated forms of infections and inflammatory diseases may be related to an inability to express suitable ligand-binding forms of the adhesion molecules, including L-selectins, but more studies are required to prove this hypothesis. We found no gender differences between these two single nucleotide polymorphisms for periodontitis. Some studies have revealed no association of certain gene polymorphisms and gender on disease susceptibility (31).

Taken together, this study demonstrated an association between the E-selectin Ser128Arg single nucleotide polymorphism and periodontitis in an Iranian population.

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References

1. Newman MG, Takei H, Carranza FA. *Clinical Periodontology*, 9th edition. Philadelphia: WB Saunders, 2002.
2. Teng YA. The role of acquired immunity and periodontal disease progression. *Crit Rev Oral Biol Med* 2003;**14:**237–252.
3. Hannigan E, Connell DP, Hannigany LA. Soluble cell adhesion molecules in gingival cervicular fluid in peridontal health and disease. *J Periodontol* 2004;**75:**546–550.
4. Michalowicz BS. Genetic and heritable risk factors in periodontal disease. *J Periodontol* 1994;**65:**479–488.
5. Zheng F, Chevalier JA, Zhang LQ, Virgil D, Ye SQ, Kwiterovich PO. An HphI polymorphism in the E-selection gene is associated with premature coronary artery disease. *Clin Gent* 2001;**59:**58–64.
6. Charles A, Travers P, Walport M, Shlomchik MJ. *Immunobiology, the Immune System in Health and Disease*, 6th edition. New York: Garland Science Publishing, 2005.
7. Imai Y. Cell adhesion molecules: selectins. Available at URL: http://www.gak.co.jp/FCCA/glycoword/LE-AO5/LEAO5E.1998, html. Accessed May 24, 2006.
8. Kornman KS. Patients are not equally susceptible to periodontitis does this change dental practice and the dental curriculum? *J Dent Educ* 2001;**65:**777–784.
9. Hodge P, Michalowicz B. Genetic predisposition to periodontitis in children and young adults. *Periodontology* 2000;**26:**113–134.
10. Schenkein HA. Finding genetic risk factors for periodontal disease. Is the clime worth the view? *Periodontology* 2002;**30:**79–90.
11. Wenzel K, Ernest M, Rohde K, Baumann G, Speer A. DNA polymorphism in adhesion molecule genes a new risk factor for early atherosclerosis. *Hum Gent* 1996;**79:**15–20.
12. Iida A, Nakamura Y. High resolution SNP map in the 55-kb region containing the selection gene family on chromosome 1q 24-q25. *J Hum Gent* 2003;**48:**150–154.
13. Rao RM, Clarke JL, Ortlepp S, Robinson MK, Landis RC, Haskard DO. The S128R polymorphism of E-selectin mediates neuraminidase resistant tethering of myeloid cells under shear flow. *Eur J Immunol* 2002;**32:**251–260.
14. Rao RM, Haskard DO, Clivelandis R. Enhanced recruitment of Th2 and CLA-negative lymphocytes by the S128R polymorphism of E-selectin. *J Immunol* 2002;**169:**5860–5865.
15. Jutila MA, Watts G, Walcheck B, Kansas GS. Characterization of a functionally important and evolutionarily well conserved epitope mapped to the short consensus repeats of E-selectin and L-selectin. *J Exp Med* 1992;**175:**1565–1573.
16. Ye SQ, Usher D, Virgil D, Zhang LQ, Yochim SE, Gupta RA. Pst1 polymorphism detects the mutation of Ser128Arginine in CD62E gene: a risk factor for coronary artery disease. *J Biomed Sci* 1999;**6:**8–21.
17. Hajilooi M, Tajik N, Sanati A, Eftekhari H, Massoud A. Association of the Phe 206 Leu allele of the selectin genes with coronary artery disease. *Cardiology* 2006;**105:**113–118.
18. Krugluger W, Nell A, Solar P, Matejke M, Boltznitulescu G. Influence of sE- selectin and L-selectin on the regulation of cell migration during chronic periodontitis. *J Periodontal Res* 1995;**30:**198–203.
19. Ghilard G, Biondi ML, Turri O, Guagnellini E, Scorza R. Ser128Arg gene polymorphism for E-selectin and severity of atherosclerotic arterial disease. *J Cardiovasc Surg (Torino)* 2004;**45:**143–147.
20. Chen HL, Hua Q, Liu RK, Yang Z. Effect of E-selectin A561C (S128R) polymorphism on blood pressure. *Zhonghua Xin Xue Guan Bing Za Zhi* 2000;**33:**603–607.
21. EL-Magadmi M, Alansari A, Tehls LS *et al*. Association of the A561C E-selectin polymorphism with systemic lupus erythematosus in 2 independent populations. *J Rheumatol* 2001;**28:**2650–2652.
22. Rafiei A, Hajilooi M, Shakib RJ, Shams S, Shikh N. Association between the Phe206Leu polymorphism of L-selectin and brucellosis. *J Med Microbiol* 2006;**55:**511–512.
23. Miller SA, Dykes DD, Poleski HF. Simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;**16:**1215.
24. Borozdenkova S, Smith J, Marshall S, Yacoub M, Rose M. Identification of ICAM-1 polymorphism that is associated with protection from transplant associated vasculopathy after cardiac transplantation. *Hum Immunol* 2001;**62:**247–255.
25. Rafiei A, Hajilooi M, Vahedi M, Shakib-Jafari R. The Ser128Arg polymorphism for E-selectin gene and brucellosis. *Infect Genet Evol* 2007;**7:**494–498.
26. Somers WS, Tang J, Shaw GD, Camphausen RT. Insights into the molecular basis of leukocyte tethering and rolling

revealed by structures of P- and E-selectin bound to SLeX and PSGL-1. *Cell* 2000;**103:**467–479.
27. Wenzel K, Stahn R, Speer A *et al*. Functional characterization of atherosclerosis-associated Ser128Arg and Leu554Phe E-selectin mutations. *Biol Chem* 1999;**380:**661–667.
28. Patel KD, Cuvelier SL, Wiehler S. Selectins: Critical mediators of leukocyte recruitment. *Semin Immunol* 2003;**14:**73–81.
29. Kinane DF, Hart TC. Genes and gene polymorphisms associated with periodontal disease. *Crit Rev Oral Biol Med* 2003;**14:**430–449.
30. Dwir O, Kansas GS, Alon R. An activated L-selectin mutant with conserved equilibrium binding properties but enhanced ligand recognition under shear flow. *J Biol Chem* 2000;**275:**18682–18691.
31. Galimberti D, Fenoglio C, Clerici R *et al*. E-selectin A561C and G98T polymorphisms influence susceptibility and course of multiple sclerosis. *J Neuroimmunol* 2005;**165:**201–205.