Journal of Clinical Laboratory Analysis 29: 191–197 (2015)

Soluble L-Selectin as an Independent Biomarker of Bronchial Asthma

Ebrahim Nadi,[1] Mehrdad Hajilooi,[2] Saeed Pajouhan,[3] and Mehran Haidari[4,5]*

[1]*Division of Pulmonary Sciences and Critical Care Medicine, Hamadan Beheshti Hospital, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*

[2]*Department of Immunology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*

[3]*Department of Internal Medicine, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran*

[4]*University of Texas Health Science Center at Houston, Houston, Texas*

[5]*Texas Heart Institute, Houston, Texas*

Background: We sought to determine the association of plasma level of soluble L-selectin (sL-selectin) and F206L polymorphism of L-selectin with asthma. *Methods:* A total of 90 asthmatic patients and 90 sex- and age-matched healthy controls were enrolled. The plasma level of sL-selectin was measured by enzyme-linked immunosorbent assay (ELISA) method. An amplification refractory mutation system polymerase chain reaction was performed to detect F206L polymorphism of L-selectin. *Results:* The mean plasma levels of sL-selectin was significantly higher in the patients with asthma than the controls (2113 ± 466 vs. 1664 ± 322 ng/ml, $P = 0.001$). Logistic regression analysis after adjustment for age, sex, and body mass index demonstrated that plasma levels of sL-selectin are an independent biomarkers for asthma (odds ratio [OR], 1.86; 95% confidence interval [95% CI], 1.42–2.24). The area under the receiver operating characteristic (ROC) curve for sL-selectin was 0.792, 95% CI (0.732–0.862), $P = 0.0001$. Individuals with the minor homozygote of F206L polymorphism of L-selectin demonstrated a higher level of sL-selectin than the major homozygous (2319 ± 732 vs. 1917 ± 453 ng/ml, $P = 0.02$). No association was found between F206L polymorphism of L-selectin with asthma. *Conclusion:* Our study suggests that plasma level of sL-selectin is an independent biomarker for asthma. J. Clin. Lab. Anal. 29:191–197, 2015.

Key words: asthma; soluble L-selectin; genetic polymorphism; receiver operating characteristic; biomarker

INTRODUCTION

It is estimated that more than 300 million people worldwide are affected with asthma (1). The prevalence of asthma in the United States had increased from 7.3% in 2001 to 8.4% in 2010, when 25.7 million persons were affected (2). Asthma is a common chronic airway disorder characterized by periods of reversible airflow obstruction. Airflow is obstructed by inflammation and airway hyperactivity (contraction of the smooth muscles surrounding the airways) in reaction to certain exposures. The airway inflammation is characterized by the influx of lymphocytes, monocytes, and eosinophils into the airway and lung (3). The process of leukocyte migration occurs through complex and highly regulated interactions between the circulating leukocytes and the vascular endothelium. Multiple families of adhesion molecules as well as specific chemoattractants and their cognate

Grant sponsor: Hamadan University of Medical Sciences; Grant number: p/16/35/119028.

*Correspondence to: Mehran Haidari, Texas Heart Institute, St. Luke's Episcopal Hospital, PO Box 20345, C1000, Houston, TX 77030. E-mail: Mehran.Haidari@uth.tmc.edu

Received 23 May 2013; Accepted 5 February 2014
DOI 10.1002/jcla.21749
Published online in Wiley Online Library (wileyonlinelibrary.com).

receptors function to stabilize these interactions and induce migration into the tissue.

L-Selectin (CD62L) plays an important role in leukocyte adhesion, where it mediates the initial capturing and tethering (4). L-selectin is constitutively expressed on all normal naive T, B cells, leukocytes, and other subset of natural killer cells (5). L-selectin contains a membrane-proximal enzymatic cleavage site that results in the rapid release of L-selectin from the cell surface following leukocyte activation. Cleavage of L-selectin results in the production of a soluble molecule that is functional in vivo (6). Cleavage is also necessary for the maintenance of appropriate cell-surface L-selectin expression levels and normal leukocyte migration (7).

There are two mutations in the L-selectin gene known in humans resulting in amino acid exchange from threonine to serine in the lectin domain at position 49 (T49S; (8)) and from phenyloalanine to leucine in the epidermal growth factor like (EGF) domain at position 206 (F20 6L; (9)). It has been suggested that mutations in the EGF domain could also be relevant for ligand binding (8, 9). It is logical to speculate that the abnormal binding function of L-selectin on lymphocytes (as a result of abnormal L-selectin molecule conformation, caused by missense mutations) could result in the impaired migration to areas of inflammation in asthma.

Both plasma levels of soluble L-selectin (sL-selectin) and F206L polymorphism of L-selectin have been implicated in inflammatory diseases (10–15). However, the roles of these two variables in asthma have not been determined. In addition, our previous studies in an Iranian population indicated that there is an association between inflammatory diseases (coronary artery diseases and brucellosis) and F206L polymorphism of L-selectin (14,15). The present study was conducted to determine the association of plasma levels of sL-selectin and F206L polymorphism of L-selectin with asthma in a population from Hamadan province of Iran. Our study demonstrated that plasma levels of sL-selectin are elevated in asthmatic patients and sL-selectin is an independent risk factor for asthma. However, based on our study, L-selectin F206L polymorphism was not associated with asthma.

MATERIALS AND METHODS

Study Population

The present study was approved by the Research and Ethics Committee of Hamadan University of Medical Services. The patients with suspected bronchial asthma before starting treatment were recruited from the outpatient clinic in Shahid Beheshti hospital in Hamadan, located in West part of Iran. In addition, previously diagnosed asthmatic patients who did not receive any drug therapy in the past 4 weeks were also selected. The inclusion criteria for all cases were bronchial asthma, and the final diagnosis was confirmed through demonstrating reversible airway obstruction. The participants were requested to fill a questionnaire for identifying their demographic characteristics, asthma history, past medical history, nocturnal and diurnal clinical signs and symptoms. In order to identify the bronchial asthma, a trained observer assessed airway reversibility, peak flowmetry, and spirometry in the asthmatic patients. At least three acceptable maneuvers from American College of Chest Physicians standards were required with the minimum of two reproducible forced expiratory volumes in 1 sec (FEV1) and forced vital capacity (FVC) maneuvers within 5% of the best measurement required for each test (16). The airway responsiveness was evaluated by a standardized protocol and the airway reversibility was evaluated by spirometry before and 15 min after inhalation of two puffs of a β-adrenergic agonist (albuterol; (17)). Equal or more than 12% increase in FEV1 (by at least 200 ml increase) was diagnostic for bronchial asthma (17). Patients were classified, based on the severity of illness, into mild persistent asthma, moderate persistent, and severe persistent. Severity is determined by a combination of physical assessment and either the peak expiratory flow rate (PEFR), or pulmonary score (18). The exclusion criteria were the presence of any inflammatory and infectious diseases in the past 2 weeks and history of recurrent infections, viral hepatitis, known collagen vascular diseases, autoimmune diseases, chronic obstructive lung disease (other than bronchial asthma), myocardial infarction/unstable angina, and being under any surgical procedures during the previous month. Also, patients who previously used inhaled steroid or systemic steroid within the past 4 weeks and those who were active smokers were excluded from the study. Ninety healthy individuals without any evidence or family history of asthma were randomly selected from the same geographical area through blood donor clinics as control group. For a period of 45 days, consecutive donors of number 5, 10, 15, and 20 in a day were asked whether they are willing to participate in the study. The blood was collected after filling the informed consent form by the donor. The donors were selected if physical examination and medical history indicated that they do not have asthma. One standardized method was used to collect blood from both healthy individuals and patients with asthma. The participants were not fasted but asked to avoid heavy and fatty meals 2 hr before blood collection. All subjects were of Iranian Caucasian origin. The body mass index (BMI) was calculated by dividing the body weight (in kilograms) by the height (in meters) squared ($BMI = weight/height^2$).

sL-Selectin Analysis

Blood samples were taken before any treatment for bronchodilator was started. Peripheral venous blood samples were taken into 3.8% 1:9 trisodium citrate containing vacuum tubes without venous occlusion. The blood samples were centrifuged immediately at 2,000 × *g* for 15 min and then the plasmas were stored in aliquots at −70°C until assayed. sL-Selectin was measured using a sandwich enzyme-linked immunoassay following the manufacturer's instructions (ELISA; Bender MedSystems, Vienna, Austria). This ELISA detects sL-selectin in concentrations ranging from 0.4 to 25 ng/ml. Plasma samples were diluted at 1/100 to 1/1,000 to obtain sL-selectin concentrations in the linear range of the assay. An anti-human sL-selectin monoclonal coating antibody is adsorbed onto microwells. Human sL-selectin present in the sample or standard binds to antibodies adsorbed to the microwells; an HRP-conjugated monoclonal anti-human sL-selectin antibody binds to human sL-selectin captured by the first antibody. Following incubation, unbound enzyme-conjugated anti-human sL-selectin is removed during a wash step and substrate solution reactive with HRP is added to the wells. A colored product is formed in proportion to the amount of human sL-selectin present in the sample. The reaction is terminated by addition of acid and absorbance is measured at 450 nm. A standard curve is prepared from seven human sL-selectin standard dilutions and human sL-selectin sample concentration determined. All assays were performed simultaneously in triplicate, without knowledge of patient group. sL-selectin was determined three times in each sample and results were expressed as mean values. The limit of detection of human sL-selectin defined as the analyte concentration resulting in an absorbance significantly higher than that of the dilution medium (mean plus 2 standard deviations) was determined to be 0.176 ng/ml (mean of eight independent assays).The coefficients of variation of the assays (CV%), according to the manufacturer, were 3 ± 5% within assays and 5 ± 10% between assays. According to the manufacturer, the average recovery ranged from 80 to 126% with an overall mean recovery of 104%. The intraassay CV% was <5%, and interassay CV% was <11% in this study, and the concentrations of the control samples fell within the ranges specified by the manufacturer. The laboratory personnel were unaware of case-control status for all tests.

Genotyping Methodology

After primary assessment, 10 ml of peripheral blood was collected from all participants. Genomic DNA was isolated via the proteinase K-buffer method (15). The polymorphism of the gene was analyzed on the basis of polymerase chain reaction (PCR) amplification. An amplification refractory mutation system PCR (ARMS-PCR) assay was performed to detect F206L polymorphism. PCR was performed with a reaction mixture containing 0.2 μl of DNA, 2 mM of each primer, 300 mM of each dNTP, 1.6 mM of $MgCl_2$, 0.3 U/μ of Taq polymerase, and 1 × KCl of buffer. Amplification was carried out using primers F5′-TATGGGCCCCAGTGTCAGT-3′, and R5″ATGGGCCCCAGTGTCAGC-3′. The products were visualized on 2% agarose gel stained with ethidium bromide.

Statistical Analysis

Statistical analysis was performed using the SPSS 14.0 software package (SPSS, Chicago, IL). All allelic and genotypic frequencies were obtained by direct counting. The Hardy–Weinberg equilibrium was tested by using a chi-square goodness of fit test. The frequencies of alleles and genotypes were compared by the Fisher exact and chi-square tests, respectively. The risk associated with sL-selectin and each L-selectin genotype in the occurrence of asthma was estimated by logistic regression analysis computing the odds ratio (OR) with the respective 95% confidence interval (CI). Receiver operating characteristic (ROC) curve was prepared by SPSS software. Correlation between sL-selectin levels and numerical variables was determined with Spearman's rank correlation. *P* values were corrected by Bonferroni correction for multiple comparisons, taking into account the number of alleles studied. Two-tailed *P* values of less than 0.05 were considered to be statistically significant. The sample size for this study was calculated by a program available from University of British Columbia, Canada (http://www.stat.ubc.ca/~rollin/stats/ssize/). To detect a minimal difference of 10% for the mean concentrations of sL-selectin between the patients and control group with a power of 80% and a value of 0.05 for α, the sample size of $n = 79$ in each group is needed.

RESULTS

No Significant Differences in the Demographic Characteristics of Participants

As shown in Table 1, there was no significant difference in the demographic characteristics of two groups with and without asthma for sex ratio, average age, and BMI.

Patients With Asthma Have Higher Plasma Levels of sL-Selectin

The distribution of frequency of plasma levels of sL-selectin in patients and control was not significantly

TABLE 1. Comparison of Male/Female Ratio, Age, and BMI Between Healthy Subject and Asthmatic Patients

	Healthy subjects, $n = 90$	Patients with asthma, $n = 90$	P-value
Male/female	55/35	56/34	0.453
Age (years)	45.3 ± 18.6	42.2 ± 18.2	0.234
BMI	25.9 ± 8.5	24.3 ± 7.9	0.632

Means ± standard deviation for age and BMI were determined for healthy subjects and patients with asthma.

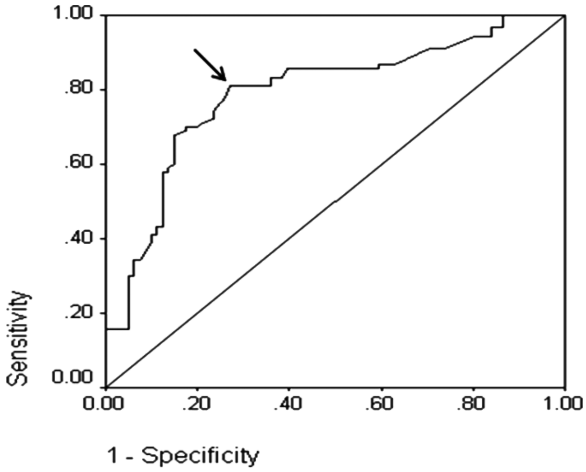

Fig. 1. Receiver operating characteristic (ROC) curve showing the performance of sL-selectin in predicting asthma. Each point on the curve represents the true-positive rate and false-positive rate associated with a particular test value (cutoff value). The area under the curve (AUC) is 0.792, 95% CI (0.732–0.862), $P = 0.0001$. An AUC value close to 1 indicates an excellent diagnostic test, a curve that lies close to the diagonal (AUC = 0.5) has no diagnostic utility. Arrow shows cutoff of 1,800 ng/ml of sL-selectin with the sensitivity and specificity of 81 and 71%, respectively.

different from a normal distribution as tested by chi-square test for goodness of fit (Fig. 1). The mean plasma level of sL-selectin was significantly higher in asthma group than control group (2113 ± 466 and 1664 ± 322 ng/ml, $P = 0.001$, respectively). Multivariable logistic regression analysis was used to control for possible confounding factors. Multivariable logistic regression analysis after adjustment for age, sex, and BMI demonstrated that plasma levels of sL-selectin is an independent risk factor for bronchial asthma (OR, 1.86; 95% CI, 1.42–2.24). ROC curve was used to determine a cutoff value for plasma level of sL-selectin. The area under the ROC curve was 0.792, 95% CI (0.732–0.862), $P = 0.0001$. As shown in Figure 1, when the cutoff value of sL-selectin was selected as 1,800 ng/ml, the sensitivity and specificity for diagnosis of asthma was 81 and 71%, respectively. On the other hand, when a value of 2,000 ng/ml was used as the cutoff point, sensitivity and specificity were 60 and

TABLE 2. L-Selectin F206L Polymorphism in Asthmatic Patients and Healthy Subjects

L-Selectin F206L polymorphism	n/Total (%) for: Control, $n = 90$	Asthma, $n = 90$	P-value
Allele			
Leucine (L)	52/180 (29)	62/180 (34)	$P = 0.315$
Phenylalanine (F)	128/180 (71)	118/180(66)	$P = 0.343$
Genotype			
Minor homozygotes (L/L)	18/180 (10.0)	30/180 (16.6)	
Major homozygotes (F/F)	94/180 (52.2)	86/180 (47.8)	$P = 0.116$
Heterozygotes (F/L)	68/180 (37.8)	64/180 (35.6)	

L-Selectin alleles and genotypes were determined in patients and control.

85.2%, respectively. No significant differences were found between males and females for the mean plasma levels of sL-selectin, age or BMI. Analysis of variances (ANOVAs) test did not show any significance differences in the mean levels of sL-selectin between the patients with mild ($n = 13$), moderate ($n = 23$), and severe ($n = 54$) form of asthma (2200 ± 264, 2159 ± 396, and 2135 ± 442 ng/ml, respectively). There was a weak but statistically significant correlation between the levels of sL-selectin and BMI ($R^2 = 0.234$; $P = 0.02$). However, no significant correlation between age and levels of sL-selectin was found.

No Significant Differences in F206L Polymorphism of L-Selectin Between the Patients and Control Groups

The allele frequencies and genotype distribution of the L-selectin F206L polymorphism in patients with asthma and healthy subjects are given in Table 2. The genotype frequencies for the control population were consistent with Hardy–Weinberg equilibrium ($X^2 = 1.167$, $P = 0.280$). As indicated in Table 2, the frequency of Leu and Phe alleles was 34 and 66% in the group with asthma and 29 and 71% in the control group with no significant difference. The results demonstrated no significant difference in the frequency of the heterozygote (Leu/Phe), and major homozygous (Phe/Phe) genotypes. The frequency of the major homozygote in asthma and control groups was 47.8 and 52.2% and for the heterozygote genotype was 35.6 and 37.8%, respectively. The statistical analysis did not show a significant difference between the frequency of minor homozygote (Leu/Leu) in the asthma and control group (16.6 vs. 10.0%). No association between asthma severity and L-selectin L206P polymorphism was found between the asthmatic patients.

Individuals With Homozygotes Leu/Leu Genotype of F206L Polymorphism of L-Selectin Have Higher Plasma Levels of sL-Selectin

To determine the association of L-selectin L206P polymorphism with plasma level of sL-selectin, the plasma levels of sL-selectin were compared between different genotypes of L206P polymorphism. The comparison of genotypes for L206F L-selectin polymorphism demonstrated that individuals with minor homozygote (Leu/Leu) genotype have higher levels of plasma sL-selectin than the major homozygote (Phe/Phe) genotypes (2319 ± 732 vs. 1917 ± 453 ng/ml, $P = 0.02$). This difference was more pronounced when we compared the two homozygotes genotypes in the patients with asthma (2619 ± 598 vs. 2121 ± 428 ng/ml, $P = 0.002$). The same analysis for healthy subjects revealed that minor homozygote genotype have higher levels of plasma sL-selectin than the major homozygote genotypes, but to a lesser extent than the asthmatic patients (2109 ± 431 vs. 1813 ± 321 ng/ml, $P = 0.04$).

DISCUSSION

We demonstrated that plasma level of sL-selectin is an independents risk factor for asthma in an Iranian population. In addition, our study indicated that individuals with minor homozygote of L-selectin F206L polymorphism (Leu/Leu) have higher plasma levels of L-selectin. However, no significant association was found between L-selectin F206L polymorphisms and asthma.

Soluble adhesion molecules are commonly formed as the result of cell surface adhesion molecule shedding due to cell stimulation. These molecules have been implicated in pathophysiology of inflammatory diseases, such as atherosclerosis and cancer (19, 20). Previous studies have demonstrated an association between an elevated plasma level of sL-selectin with inflammatory, autoimmune, and systemic diseases, such as type I diabetes, systemic lupus erythematosus, ischemic stroke, and acute leukemia (10–13).

There has been controversy over the function of soluble adhesion molecules, including sL-selectin in the inflammatory diseases. Soluble adhesion molecules have been shown to both inhibit and enhance different aspects of the inflammatory process. L-Selectin-dependent leukocyte attachment is completely inhibited at the presence of sL-selectin (21). In addition, it has been demonstrated that in patients with acute leukemia, sL-selectin inhibits blast cell adhesion to activated endothelium (13). It has been demonstrated that sL-selectin attenuated TNF-α-induced leukocytes adherence to endothelial cells (22).

We are the first group to demonstrate an association between elevated levels of sL-selectin and asthma. L-Selectin plays a critical role in lymphocyte migration to the lung during an allergic inflammatory response (23–25). It has been reported that endothelial sulfated sialyl Lewis × glycans, a putative L-selectin ligands, are preferentially expressed in bronchial asthma (26). In addition, expression of L-selectin by T cells is increased in asthmatic individual (27).

It has been shown that L-selectin mAbs, antibody against L-selectin ligand or low-molecular weight selectin antagonists, are therapeutic in the animal models of allergic bronchoconstriction (28–30). L-Selectin null mice that are sensitized to ovalbumin are protected from the development of airway hyperresponsiveness of asthma after ovalbumin challenge (31, 32). In clinical trials, multiple doses of a synthetic pan-selectin antagonist given by aerosol indicated some protection against the late airway responses (33). Furthermore, Huang et al. demonstrated that long-term immunotherapy in children with asthma reduces serum level of L-selectin (34).

Further investigations are needed to determine the functional role of sL-selectin in the pathophysiology of asthma. The elevated plasma levels of sL-selectin may protect asthmatic patients by inhibiting the adherence of leukocytes to endothelium of bronchial airways or it may be just a bystander that can be used as a biomarker for asthma. In recent years, it has become increasingly accepted that pulmonary function testing has its limitations. Spirometry can identify a broad spectrum of asthmatics, but it is incapable of discerning the various subtypes of disease, and therefore which individuals will respond to normal treatment regimes. Based on our ROC analysis, we speculate that sL-selectin has the potential of being used as a biomarker for diagnose and monitoring of asthma. Future studies will shed light on the usefulness of sL-selectin as a noninvasive biomarker for diagnosis and monitoring of asthma that can complement spirometry.

The levels of sL-selectin have been suggested to be influenced by F206L polymorphism of L-selectin. In agreement with our study, Russell et al. demonstrated that sL-selectin is associated with L-selectin F206L polymorphisms in the EGF-like domain of L-selectin in SLE patients (11). F206L polymorphism of L-selectin has been implicated in inflammatory diseases. Rafiei et al. showed that the 206 Leu allele was more frequent in patients with brucellosis in comparison to healthy controls (14). In another observation by Hajilooi et al., a significantly increased frequency of the 206 Leu mutant allele was observed in patients with coronary artery disease compared to the controls (15).

Study Limitations

Our study did not show any signification association between F206L polymorphism of L-selectin and asthma. The sample size for this study was calculated for the

detection of a minimal difference of 10% for the mean concentrations of sL-selectin between the patients and control group. Therefore, the results for L-selectin F206L polymorphisms should be interpreted with caution. The frequency of minor homozygote (Leu/Leu) in the asthma group was greater than the controls (16.6.0 vs. 10.0%). However, the statistical analysis did not show a significant difference. This can be attributed to the low number of individuals with Leu/Leu genotype. We are currently endeavoring to increase the sample size to further study these findings. In conclusion, our study demonstrated that plasma level of sL-selectin is higher in asthma and is an independent risk factor for this disease.

REFERENCES

1. Masoli M, Fabian D, Holt S, et al. The global burden of asthma: Executive summary of the GINA Dissemination Committee report. Allergy 2004;59:469–478.
2. Akinbami LJ, Moorman JE, Bailey C, et al. Trends in asthma prevalence, health care use, and mortality in the United States, 2001–2010. NCHS Data Brief 2012;94:1–8.
3. Bradley BL, Azzawi M, Jacobson M, et al. Eosinophils, T-lymphocytes, mast cells, neutrophils, and macrophages in bronchial biopsy specimens from atopic subjects with asthma. Comparison with biopsy specimens from atopic subjects without asthma and normal control subjects, and relationship to bronchial hyperresponsiveness. L Allergy Clin Immunol 1991;88:661–674.
4. Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994;84:2068–2101.
5. Steeber DA, Green NE, Sato S, Tedder TF. Humoral immune responses in L-selectin deficient mice. J Immunol 1996;157:4899–4907.
6. Kahn J, Ingraham RH, Shirley F, Migaki GI, Kishimoto TK. Membrane proximal cleavage of L-selectin: Identification of the cleavage site and a 6-kD transmembrane peptide fragment of L-selectin. J Cell Biol 1994;125:461–470.
7. Venturi GM, Tu L, Kadono T, et al. Leukocyte migration is regulated by L-selectin endoproteolytic release. Immunity 2003;19:713–724.
8. Tedder T, Isaacs CM, Ernst TJ, Demetri GD, Adler DA, Disteche CM. Isolation and chromosomal localization of c DNAs encoding a novel human lymphocyte cell surface molecule LAM-1. J Exp Med 1989;170:123–133.
9. Wenzel K, Ernst M, Rohde K, Baumann G, Speer A. DNA polymorphisms in adhesion molecule genes—A new factor for early atherosclerosis. Hum Genet 1996;97:15–20.
10. Kretowski A, Gillespie KM, Bingley PJ, Kinalska I. Soluble L-selectin levels in type I diabetes mellitus: A surrogate marker for disease activity? Immunology 2000;99:320–325.
11. Russell AI, Cunninghame Graham DS, Chadha S, et al. No association between E- and L-selectin genes and SLE: Soluble L-selectin levels do correlate with genotype and a subset in SLE. Genes Immun 2005;6:422–429.
12. Wei YS, Lan Y, Meng LQ, Nong LG. The association of L-selectin polymorphisms with L-selectin serum levels and risk of ischemic stroke. J Thromb Thrombolysis 2011;32:110–115.
13. Spertini O, Callegari P, Cordey AS, et al. High levels of the shed form of L-selectin are present in patients with acute leukemia and inhibit blast cell adhesion to activated endothelium. Blood 1994;84:1249–1256.
14. Rafiei A, Hajilooi M, Shakib RJ, Shams S, Sheikh N. Association between the Phe206Leu polymorphism of L-selectin and brucellosis. J Med Microbiol 2006;55:511–516.
15. Hajilooi M, Tajik N, Sanati A, Eftekhari H, Massoud A. Association of the Phe206Leu allele of the L-selectin gene with coronary artery disease. Cardiology 2006;105:113–118.
16. Snider GL, Woolf CR, Kory RC. Criteria for the assessment of reversibility in airway obstruction: Report of the committee on Emphysema, American College of Chest Physicians. Chest 1974;65:552–553.
17. Miller MR, Hankinson J, Brusasco V, et al. ATS/ERS task force. Standardization of spirometery. Eur Respir J 2005;26:319–338.
18. National Heart, Lung, and Blood Institute. National Asthma Education Program, expert panel report. Guidelines for the diagnosis and management of asthma (NIH Pub no 97–4051). Bethesda, MD: NIH Publication; 1997.
19. Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. Clin Chim Acta 2006:368;33–47.
20. van Kilsdonk JW, van Kempen LC, van Muijen GN, Ruiter DJ, Swart GW. Soluble adhesion molecules in human cancers: Sources and fates. Eur J Cell Biol 2010;89:415–427.
21. Schleiffenbaum B, Spertini O, Tedder TF. Soluble L-selectin is present in human plasma at high levels and retains functional activity. J Cell Biol 1992;119:229–238.
22. Ferri LE, Pascual J, Seely AJ, Chaudhury P, Christou NV. Soluble L-selectin attenuates tumor necrosis factor-alpha-mediated leukocyte adherence and vascular permeability: A protective role for elevated soluble L-selectin in sepsis. Crit Care Med 2002;30:1842–1847.
23. Keramidaris E, Merson TD, Steeber DA, Tedder TF, Tang ML. L-selectin and intercellular adhesion molecule 1 mediate lymphocyte migration to the inflamed airway/lung during an allergic inflammatory response in an animal model of asthma. J Allergy Clin Immunol 2001;107:734–738.
24. Tang ML, Fiscus LC. Important roles for L-selectin and ICAM-1 in the development of allergic airway inflammation in asthma. Pulm Pharmacol Ther 2001;14:203–210.
25. Symon FA, McNulty CA, Wardlaw AJ. P- and L-selectin mediate binding of T cells to chronically inflamed human airway endothelium. Eur J Immunol 1999;29:1324–1333.
26. Toppila S, Paavonen T, Laitinen A, Laitinen LA, Renkonen R. Endothelial sulfated sialyl Lewis x glycans, putative L-selectin ligands, are preferentially expressed in bronchial asthma but not in other chronic inflammatory lung diseases. Am J Respir Cell Mol Biol 2000:23:492–498.
27. Arifhodzic NA, Mahmoud FF, Abul HT, et al. Major lymphocyte populations and T-cell expression of ICAM-1 and L-selectin adhesion molecules in Kuwaitis with asthma and rhinitis. Arch Environ Occup Health 2005;60:243–247.
28. Rosen SD, Tsay D, Singer MS, Hemmerich S, Abraham WM. Therapeutic targeting of endothelial ligands for L-selectin (PNAd) in a sheep model of asthma. Am J Pathol 2005;166:935–944.
29. Abraham WM, Ahmed A, Sabater JR, et al. Selectin blockade prevents antigen-induced late bronchial responses and airway hyperresponsiveness in allergic sheep. Am J Respir Crit Care Med 1999;159:1205–1214.
30. Oostingh GJ, Ludwig RJ, Enders S, et al. Diminished lymphocyte adhesion and alleviation of allergic responses by small-molecule- or antibody-mediated inhibition of L-selectin functions. J Invest Dermatol 2007;127:90–97.
31. Fiscus LC, Van Herpen J, Steeber DA, Tedder TF, Tang ML. L-selectin is required for the development of airway hyperresponsiveness but not airway inflammation in a murine model of asthma. J Allergy Clin Immunol 2001;107:1019–1024.

32. Royce SG, Lee M, Tang ML. The contribution of L-selectin to airway hyperresponsiveness in chronic allergic airways disease. J Asthma Allergy 2010;28;9–17.
33. Beeh KM, Beier J, Meyer M, Buhl R, Zahlten R, Wolff G. Bimosiamose, an inhaled small-molecule pan-selectin antagonist, attenuates late asthmatic reactions following allergen challenge in mild asthmatics: A randomized, double-blind, placebo-controlled clinical cross-over-trial. Pulm Pharmacol Ther 2006;19:233–241.
34. Huang JL, Ou LS, Tsao CH, Chen LC, Kuo ML. Reduced expression of CD69 and adhesion molecules of T lymphocytes in asthmatic children receiving immunotherapy. Pediatr Allergy Immunol 2002;13:426–433.