

Soluble CD40 Ligand and Soluble P-Selectin in Allergic Asthma Patients During Exercise-Induced Bronchoconstriction

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■ Abstract

Background: The interactions between CD40 and its ligand, CD40L, control humoral and cell-mediated immune responses. CD40 ligation may promote asthma-associated inflammatory responses in the airways. Many reports confirm the inflammatory basis of exercise-induced bronchoconstriction (EIB) in asthmatics.

Methods: The study was conducted in a group of 19 asthmatic patients (11 with EIB, 8 without EIB) and 8 healthy volunteers. We analyzed the changes in plasma concentrations of soluble CD40 ligand (sCD40L) and soluble P-selectin (sP-selectin) induced by intensive exercise. We also studied possible correlations with the results of measurements commonly associated with asthmatic inflammation.

Results: The study revealed statistically significant higher baseline concentrations of sCD40L—but not sP-selectin—in the group of asthmatics with EIB than in those without. In the asthmatic patients with EIB, sCD40L and sP-selectin concentrations increased significantly 30 minutes after exercise and returned to baseline 24 hours after exercise. Baseline concentrations of sCD40L correlated with baseline sP-selectin or fractional exhaled nitric oxide concentration (FE_{NO}), an increase in sP-selectin 30 minutes after exercise, and changes in FE_{NO} or bronchial hyperresponsiveness 24 hours after exercise. A statistically significant correlation between an increase in sCD40L concentrations 30 minutes after exercise and an increase in FE_{NO} 24 hours after exercise or baseline eosinophil cationic protein was observed.

Conclusion: After exercise in the group of allergic asthmatics with EIB, upregulation of CD40L by increased expression of inflammatory molecules and improved sensitivity of CD40-responsive cell types to the effects of proinflammatory cytokines may play an important role in the increased airway inflammation observed after postexercise bronchoconstriction.

Key words: Asthma. Exercise-induced bronchoconstriction. sP-selectin. sCD40 ligand. Airway inflammation.

■ Resumen

Antecedentes: Las interacciones entre la CD40 y su ligando, el CD40L, controlan las respuestas inmunitarias humorales y las mediadas por células. La unión de CD40 puede estimular las respuestas inflamatorias relacionadas con el asma en las vías respiratorias. No son pocos los informes que confirman que la broncoconstricción inducida por el ejercicio (BIE) en los asmáticos tiene una base inflamatoria.

Métodos: El estudio se realizó en un grupo de 19 pacientes asmáticos (11 con BIE y 8 sin BIE) y 8 voluntarios sanos. Analizamos los cambios en las concentraciones plasmáticas del ligando de CD40 soluble (sCD40L) y de la P-selectina (sP-selectina) inducidos por el ejercicio intenso y las posibles correlaciones con los resultados de las mediciones frecuentemente relacionadas con la inflamación asmática.

Resultados: El estudio reveló que existían concentraciones iniciales estadísticamente significativas y más altas de sCD40L—pero no de sP-selectina—en el grupo de asmáticos con BIE que en los que no presentaban BIE. En los pacientes asmáticos con BIE, las concentraciones de sCD40L y sP-selectina aumentaron significativamente 30 minutos después del ejercicio y volvieron a los valores iniciales 24 horas después del ejercicio. Las concentraciones iniciales de sCD40L se correlacionaban con los valores iniciales de sP-selectina o concentración fraccionada de óxido nítrico exhalado (FE_{ON}), con un aumento en la sP-selectina 30 minutos después del ejercicio y con cambios en la FE_{ON} o la hiperreactividad bronquial 24 horas después del ejercicio. También se observó una correlación estadísticamente significativa entre un aumento en las concentraciones de sCD40L, 30 minutos después del ejercicio y un aumento de FE_{ON}, 24 horas después del ejercicio o la proteína catiónica del eosinófilo inicial.

Conclusión: La regulación de CD40L por el aumento de la expresión de las moléculas inflamatorias y el incremento de la sensibilidad de los tipos celulares sensibles a CD40 para los efectos de las citocinas proinflamatorias puede tener un papel importante en el aumento de la inflamación de las vías respiratorias que se observa tras la broncoconstricción inducida por ejercicio, en el grupo de asmáticos alérgicos con BIE.

Palabras clave: Asma. Broncoconstricción inducida por el ejercicio. sP-selectina. Ligando sCD40. Inflamación de las vías respiratorias.

Introduction

Interaction between CD40 and its ligand, CD40L, is an important pathway for B-cell and T-cell cooperation and function. CD40L is a type 2 transmembrane protein classified as a member of the tumor necrosis factor family (TNF). It is expressed on activated CD4⁺ T cells, activated CD8⁺ T cells, eosinophils, mast cells, basophils, natural killer cells, and activated dendritic cells [1]. Many reports have indicated that interactions between CD40 and CD40L control the development of humoral and cell-mediated immune responses [2,3]. Ligation of CD40 expressed on airway epithelial cells upregulates the expression of inflammatory mediators, including interleukin (IL)-8, RANTES, monocyte chemoattractant protein-1, and adhesion molecules. These findings suggest that CD40 ligation may promote inflammatory responses in the airways [2,3].

Exercise-induced bronchoconstriction (EIB) is a common clinical problem occurring in most patients with chronic asthma and in nearly half of the allergic population. Despite the wide prevalence and clinical significance of EIB, the mechanisms responsible for it have yet to be established [4]. Cooling of the airway during exercise dries the mucosa and increases osmolarity. This leads to mast cell degranulation and mediator release, which are responsible for the symptoms of EIB. A late-phase response to exercise can be observed in some patients, in whom increased inflammation of the airway could also be present [5].

Findings related to the participation of inflammatory mediators in either the induction or maintenance of EIB are conflicting [6]. We have already analyzed the possible role of pulmonary vascular endothelium in the pathogenesis of EIB, particularly in the inflammatory basis of this condition and airway remodeling [7].

Therefore, we compared soluble CD40L (sCD40L) levels

in the plasma of allergic asthma patients during an exercise test with other measurements and laboratory tests used in the diagnosis of the asthma inflammatory process (exhaled nitric oxide, baseline lung function, nonspecific bronchial hyperreactivity, elevated levels of serum immunoglobulin [Ig] E, and eosinophil cationic protein [ECP]) and with platelet activation markers.

Methods

Subjects

The study population was composed of 19 patients with mild allergic asthma (11 with EIB, 8 without EIB) diagnosed according to the criteria of the Global Initiative for Asthma [8]. The presence of an allergy to common allergens (house dust mite, trees, weeds, grasses, cat, *Alternaria*, or *Cladosporium*) was demonstrated in all patients by positive skin prick tests. Patient characteristics are shown in Table 1. All patients were stable and had been free from acute exacerbations and respiratory tract infections for the 4 weeks before the study. Patients with other factors that could change fractional exhaled nitric oxide (FE_{NO}) levels (except for asthma, features of atopy, or allergic rhinitis) were excluded from the study. Before the study, patients were allowed to take short-acting β_2 -agonists. Asthmatic patients who had been treated with inhaled corticosteroids in the previous 3 months were excluded from the study.

The control group contained 8 healthy volunteers, all of whom underwent FE_{NO}, flow/volume spirometry, and prick tests with common inhaled allergens. All healthy volunteers were free of chronic or recurrent respiratory symptoms, and had no history of significant pulmonary disease. They were nonatopic and had a forced expiratory volume in 1 second

Table 1. Characteristics of Study Subjects and Healthy Volunteers^a

Characteristics	Patients With EIB	Patients Without EIB	Differences Between Groups of Asthma Patients	Healthy Volunteers
Number of patients	11	8		8
Sex, F/M	7/4	5/3		5/3
Age, y	33.45 (7.80)	33.25 (7.04)	<i>P</i> = .95	29.87 (7.05)
Duration of symptoms, y	4.2 (3.5)	3.6 (2.3)	<i>P</i> = .67	
Baseline FEV ₁ , % pred	86.72 (9.10)	92.87 (5.96)	<i>P</i> = .11	98.37 (4.45) ^{b,c}
PC ₂₀ histamine FEV ₁ , mg/mL	1.32 (0.93)	1.60 (1.16)	<i>P</i> = .56	
Serum ECP, μ g/L	14.56 (2.51)	11.11 (2.11)	<i>P</i> = .005	2.88 (0.84) ^{b,c}
Serum total IgE, kU/L	270 (128)	191 (74)	<i>P</i> = .13	39 (12) ^{b,c}
Baseline FE _{NO} , ppB	84 (49)	60 (36)	<i>P</i> = .27	15 (5) ^{b,c}
Baseline sCD40 ligand, pg/mL	522.99 (395.11)	161.06 (89.00)	<i>P</i> = .021	146.01 (70.88) ^b
Baseline sP-selectin, ng/mL	46.24 (13.27)	52.71 (22.41)	<i>P</i> = .44	35.27 (7.23) ^{b,c}

Abbreviations: ECP, eosinophil cationic protein; EIB, exercise-induced bronchoconstriction; FE_{NO}, fractional exhaled nitric oxide concentration; FEV₁, forced expiratory volume in 1 second; Ig, immunoglobulin; PC₂₀ histamine FEV₁, provocative concentration of histamine that caused a 20% fall in FEV₁

^a Data are presented as mean (SD)

^b Values significantly different from patients with EIB, *P* < .05

^c Values significantly different from patients without EIB, *P* < .05

(FEV₁) greater than 80% predicted. They had been free of respiratory tract infection during the 2 months before the study and from other significant illnesses known to affect FE_{NO} measurements.

Asthma patients and healthy volunteers were nonsmokers and during the previous year had not been passive smokers.

The study protocol was approved by the ethics committee of the Medical University of Bialystok (number of agreement R-I-003/163/2005). Informed consent was obtained from every patient enrolled in the study.

Measurements

Baseline spirometry was performed using a MasterScreen Pneumo PC spirometer (Jaeger, Hoechberg, Germany) according to the criteria of the American Thoracic Society [9]. FEV₁ was evaluated. Before the examination, the patients did not take any medications that could change spirometry results. The highest value of 3 technically satisfactory attempts was used.

A nonspecific bronchial provocation test (BPT) with histamine was carried out according to the method described by Ryan et al [10]. Provocation was performed using a De Villbiss nebulizer 646 (Viasys Healthcare GmbH, Hoechberg, Germany) at an air pressure of 0.15 MPa linked to a Rosenthal-French dosimeter (Laboratory For Applied Immunology c/o Richard R. Rosenthal M.D, Baltimore, USA). The results were presented as

PC₂₀ FEV₁, the concentration of histamine that causes a decrease in FEV₁ of exactly 20% compared with initial values.

The exercise test was performed on a bicycle ergometer for 9 minutes with a fixed workload adjusted to increase the heart rate to 85% of the maximum predicted for the age of each patient [11]. Serial FEV₁ measurements were made 5 minutes before exercise and at 5, 10, 15, 20, 30, 60 minutes, and 24 hours after exercise. Those patients whose maximum decrease in FEV₁ was greater than 15% from baseline were considered to have EIB. Blood samples were collected before, 30 minutes after, and 24 hours after the exercise test.

FE_{NO} was measured in all of the asthma patients by the chemiluminescence technique using a Sievers 280i Nitric Oxide Analyzer (Boulder, Colorado, USA). Measurements were performed at an expiratory flow of 50 mL/s. To produce a stable nitric oxide (NO) level for 3 seconds, the duration of exhalation lasted at least 6 seconds. Repeated measurements were performed until 3 values varied by less than 10%. The mean value of the 3 measurements was taken as the final FE_{NO} level [12]. Serial FE_{NO} measurements were made after exercise at 10 minutes, 30 minutes, and 24 hours.

Serum total IgE concentration and serum ECP concentration were measured using ImmunoCAP™ Technology (Pharmacia Diagnostics, Uppsala, Sweden).

The plasma concentrations of the sCD40 ligand and soluble

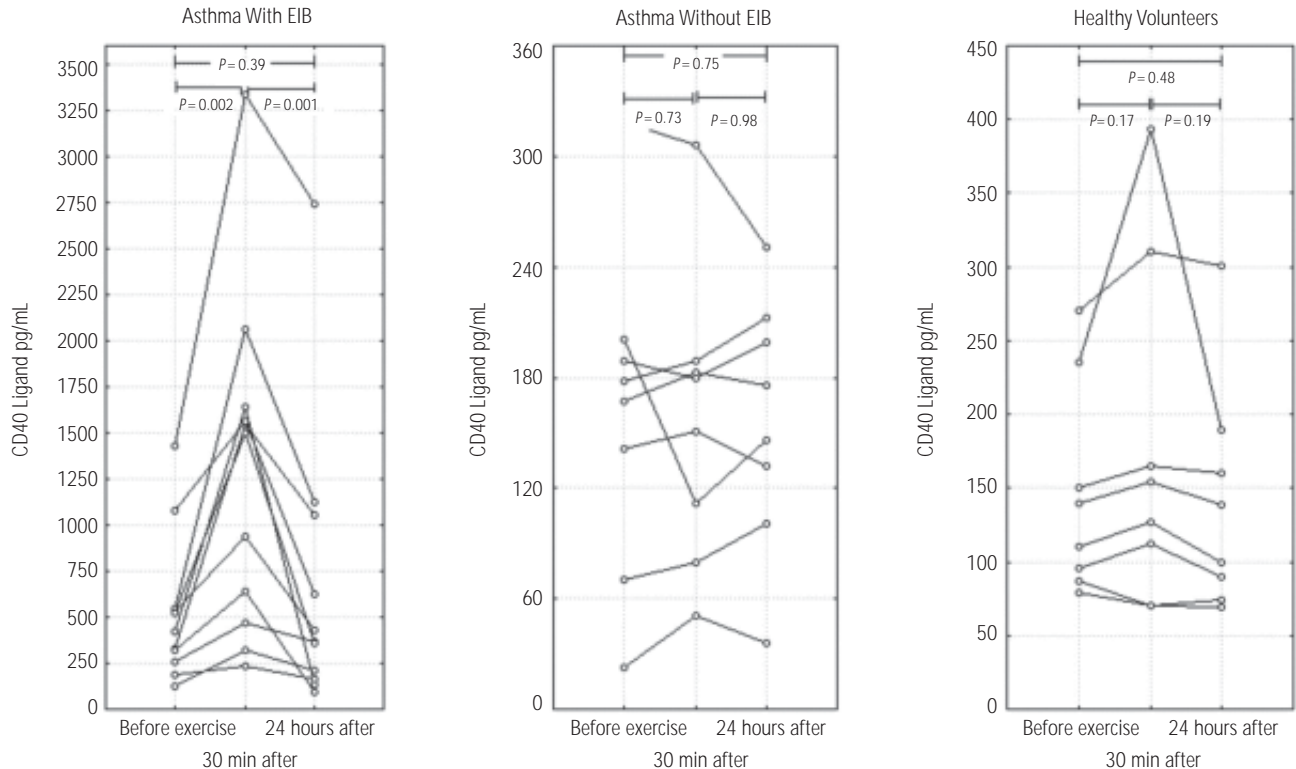


Figure 1. Concentrations of plasma sCD40 ligand at rest, and subsequent changes during the exercise test in groups of patients with asthma and healthy volunteers.

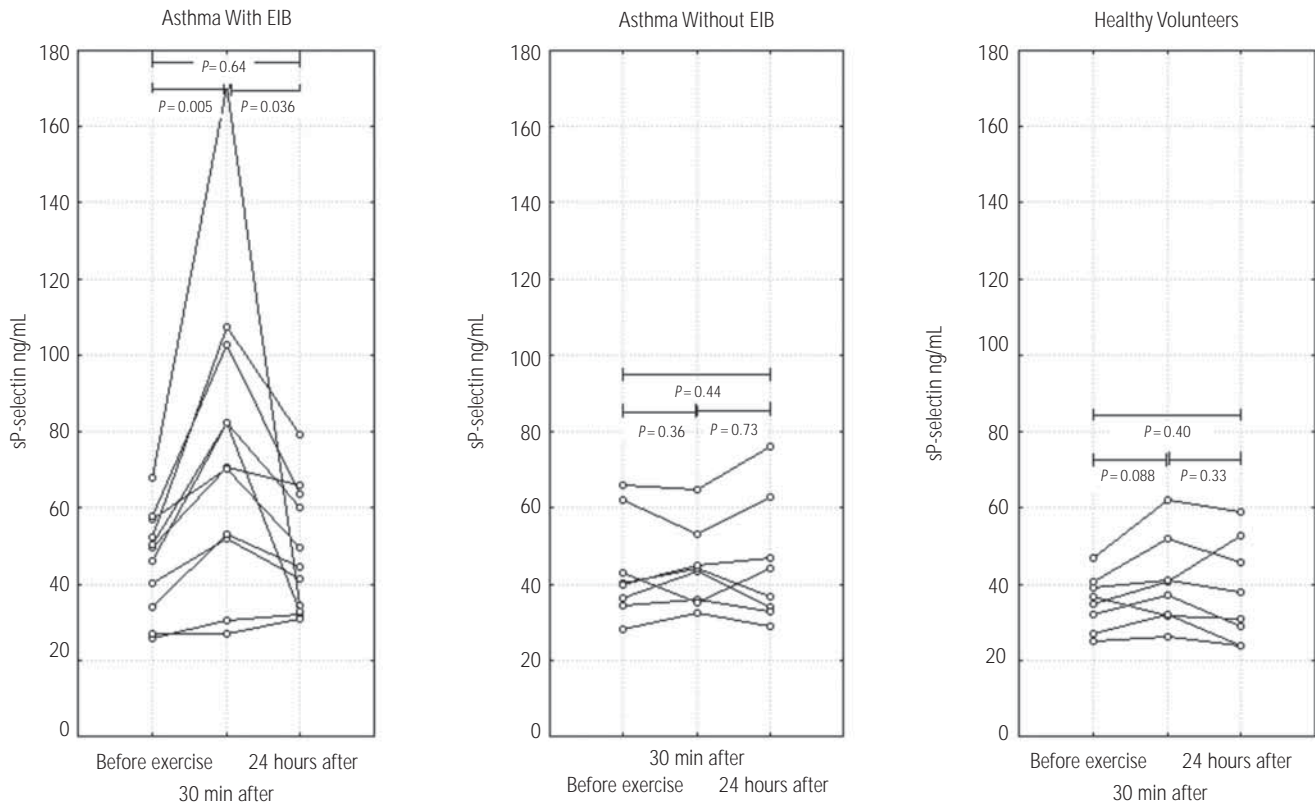


Figure 2. Changes in the plasma concentrations of sP-selectin induced by intensive exercise in the studied groups of asthmatics and healthy volunteers.

P-selectin (sP-selectin) were determined using an enzyme-linked immunosorbent assay (R&D Systems, Wiesbaden-Nordenstadt, Germany).

Statistical Analysis

Statistical analyses were completed using the *t* test (Statistica computer programme, StatSoft Poland, Cracow, Poland). All values were expressed as the means (SD), and *P* values < .05 were considered significant. The relationship between the parameters studied was evaluated using Pearson's linear correlation coefficient.

Results

Exercise-Induced Changes In Baseline Levels Of Studied Parameters

The pre-exercise concentration of sCD40L was significantly higher in the group of asthmatics with EIB than in the group of asthmatic patients without EIB and healthy volunteers (patients with EIB, 522.99 pg/mL [395.11] vs. patients without EIB, 161.06 [89.00], *P* = .021; patients with EIB vs. healthy volunteers, 146.01 [70.88], *P* = .016; patients without EIB vs. healthy volunteers *P* = .71). In the group of patients with EIB, a statistically significant increase in sCD40L was observed 30

minutes after exercise (1260.2 pg/mL [831.9]). Twenty-four hours after exercise, the concentration of sCD40L had returned to the baseline value (655.8 pg/mL [749.2]). There were no significant changes in the group of asthma patients without EIB and healthy volunteers before and after exercise (Figure 1).

The concentrations of sP-selectin at rest in the respective groups of asthmatic patients did not reveal any statistically significant differences (patients with EIB 46.24 ng/mL [13.27] vs. patients without EIB 52.71 [22.41], *P* = .44). In both groups of asthmatic patients, baseline sP-selectin concentrations were significantly higher than in healthy volunteers (patients with EIB vs. healthy volunteers: 35.27 [7.23], *P* = .049; patients without EIB vs. healthy volunteers: *P* = .048). In the group of asthma patients with EIB, a statistically significant increase in the concentration of sP-selectin 30 minutes after exercise was observed (75.61 [40.31]). Twenty-four hours after exercise, the concentration of sP-selectin returned to the baseline value (49.8 [15.8]). No significant differences in the concentrations of sP-selectin after exercise in asthmatic patients without EIB and healthy volunteers were observed (Figure 2).

Correlations Between Baseline Concentrations of sCD40L and sP-Selectin and Other Studied Parameters in the Group of Asthmatics With EIB

There was a significantly positive correlation between concentrations of sCD40L and either sP-selectin or FE_{NO}

Table 2. Correlations Between the Rest Concentrations of sCD40 Ligand and sP-Selectin and Other Studied Parameters in the Group of Asthmatic Patients With EIB

Studied Parameters	Increase in sCD40L 30 Minutes After Exercise	sP-selectin	Increase in sP-Selectin 30 Minutes After Exercise	ECP	IgE	FE _{NO}	Increase in FE _{NO} 24 Hours After Exercise	PC ₂₀ FEV ₁ Histamine	Decrease in PC ₂₀ FEV ₁ Histamine 24 Hours After Exercise
Baseline sCD40L	r=0.59 P=.054	r=0.77 P=.005	r=0.51 P=.01	r=0.47 P=.13	r=0.53 P=.09	r=0.92 P=.00005	r=0.63 P=.035	r=-0.28 P=.4	r=-0.66 P=.02
Baseline sP-selectin	r=0.57 P=.63P		r=0.77 P=.004	r=0.67 P=.02	r=0.44 P=.17	r=0.7 P=.016	r=0.42 P=.19	r=0.069 P=.84	r=-0.24 P=.46

before exercise and between baseline sP-selectin and either ECP or FE_{NO} before exercise. There was a significant positive correlation between concentrations of sCD40L at rest and either the increase in sCD40L or sP-selectin 30 minutes after exercise and an increase in FE_{NO} and decrease in PC₂₀ FEV₁ 24 hours after exercise. We observed a statistically significant positive correlation between baseline sP-selectin concentration and the increase in sP-selectin 30 minutes after exercise in asthmatics with EIB (Table 2).

There were no statistically significant correlations between baseline concentrations of sCD40L and sP-selectin or other studied parameters in the group of asthmatic patients without EIB or the healthy volunteers.

Correlations Between Changes in sCD40L and sP-Selectin After Exercise and Other Parameters Studied

Twenty-four hours after the exercise test in the group of asthmatics with EIB, we observed a statistically significant increase in FE_{NO} (before exercise, 84 ppB [49]; 24 hours after

exercise, 100 [56], P=.0006) and bronchial hyperresponsiveness (BHR) to histamine (PC₂₀ FEV₁ before exercise, 1.32 mg/mL ± 0.93; 24 hours after exercise, 1.17 [0.97], P=.029). Such changes were not observed in the group of asthmatic patients without EIB (FE_{NO} before exercise, 60 ppB [36]; 24 hours after exercise, 63 [35], P=.09; PC₂₀ FEV₁ before exercise, 1.60 mg/mL [1.16]; 24 hours after exercise, 1.57 [1.15], P=.38). We did not detect significant changes in FEV₁ 24 hours after exercise in either group of asthmatics.

We observed a statistically significant correlation between the increase in sCD40L concentrations 30 minutes after exercise and the increase in FE_{NO} 24 hours after exercise or the increase in sP-selectin 30 minutes after exercise. We also observed a statistically significant correlation between the increase in sCD40L 30 minutes after exercise and baseline ECP, although not between the increase in sP-selectin concentrations 30 minutes after exercise (Table 3).

No changes in the mean plasma concentrations of sCD40L or sP-selectin were found within 30 minutes after nonspecific bronchial provocation test with histamine (data not shown).

Table 3. Correlations Between the Increase in sCD40 Ligand and sP-selectin 30 Minutes After Exercise and Other Studied Parameters in the Group of Asthmatic Patients With EIB

Studied Parameters	Increase in sP-selectin 30 Minutes After Exercise	ECP	FE _{NO}	Increase in FE _{NO} 24 Hours After Exercise	PC ₂₀ FEV ₁ Histamine	Decrease in PC ₂₀ FEV ₁ Histamine 24 Hours After Exercise
Increase in sCD40L 30 minutes after exercise	r=0.78 P=.004	r=0.66 P=.02	r=0.46 P=.14	r=0.71 P=.014	r=-0.23 P=.49	r=-0.48 P=.12
Increase in sP-selectin 30 minutes after exercise		r=0.49 P=.12	r=0.55 P=.075	r=0.46 P=.15	r=0.01 P=.96	r=0.28 P=.40

Discussion

CD40 ligand (CD40L), also known as CD154, a member of the TNF superfamily, is a multifunctional ligand. Interaction between CD40 and CD40L is critical to the control of thymus-dependent humoral immunity and cell-mediated immune responses [1,13-15]. The major component of the contact-dependent signal leading to B-cell activation is CD40L, which stimulates B-cell secretion of immunoglobulin isotypes in the presence of cytokines. Activated T cells can express both a membrane-associated and a soluble form of CD40L (sCD40L) [16]. The receptor for CD40L is CD40, a member of the TNF receptor superfamily. Interaction between CD40L and CD40 not only induces proliferation and isotype switching in B lymphocytes, but also mediates a wide variety of other immune and inflammatory responses [1,13,14]. CD40 signaling has been linked to pathogenic processes of chronic inflammatory diseases [15]. The loss of interaction between CD40 and CD40L can result in impairment of T-lymphocyte functions, B-lymphocyte differentiation, and monocyte functions [16]. The interactions between CD40-responsive airway epithelial cells and CD40 ligand+ leukocytes (such as activated T cells, eosinophils, and mast cells) modulate asthma-associated airway inflammation. CD40 ligation enhances the response of airway epithelial cells to the effects of proinflammatory cytokines (for example TNF- α and IL-1 β) present in the local microenvironment [17].

CD40L is expressed primarily on activated CD4+ T cells; however, platelets, vascular endothelial cells, smooth muscle cells, macrophages, basophils, eosinophils, monocytes, dendritic cells, fibroblasts, and mast cells also express CD40L. Cytokine stimulation (IL-1 β , TNF- α , or INF- γ) can increase surface levels and de novo synthesis of CD40L in certain cell types [18].

Kowal et al [19] revealed that, in asthmatic patients, bronchial allergen challenge results in activation of peripheral blood platelets. The correlation between the changes in platelet activation markers and the development of a late asthmatic reaction suggests that platelet activation within circulation plays a critical role in the development of chronic allergic inflammation.

Our results show that the plasma kinetics of sCD40L is similar to that of sP-selectin, an important marker of platelet activation and suggest that platelets could be an important source of intravascular sCD40L in humans. This is consistent with the findings of previous studies [19,20-22].

The mechanism for EIB is probably a combination of airway heat and water loss, since these events occur together physiologically and alteration of either variable intensifies severity [23]. The release of a variety of mediators from mast cells (including histamine, leukotrienes, prostaglandins, thromboxanes, and a platelet-activating factor) is induced by intensive exercise. Findings related to the participation of inflammatory mediators in either the maintenance or induction of EIB are conflicting, although many reports demonstrate that EIB could have an inflammatory basis [24]. We have already shown that changes in the function of the pulmonary endothelium occur during EIB and these changes may influence inflammation and remodeling of the airway in asthmatic patients [7].

This study was designed to clarify the possible role of sCD40L in the pathogenesis of EIB, particularly in the inflammatory basis of this condition. Our results show that significant upregulation of sCD40L in asthmatic patients during postexercise bronchoconstriction is associated with increased expression of other markers of airway inflammation (an increase in exhaled NO, which has become a more and more appreciable criterion for the evaluation of airway inflammation 24 hours after exercise [25]). In the group of asthmatics with EIB, elevated baseline sCD40L levels can be observed. These correlate with an increase in selected markers of airway inflammation, upregulation of sCD40L, and an increase in platelet activation markers after exercise. In the group of allergic asthma patients, upregulation of CD40L after exercise—through the increased expression of inflammatory molecules and enhancement of the sensitivity of CD40-responsive cell types to the effects of the proinflammatory cytokines—may play an important role in the increased airway inflammation observed after postexercise bronchoconstriction. This process is not observed in asthmatics, in whom postexercise bronchoconstriction does not occur. Proper anti-inflammatory treatment and prevention of postexercise bronchoconstriction may play a crucial role in limiting the effect of EIB on airway inflammation.

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