

Short communication

IgE-mediated downregulation of L-selectin (CD62L) on lymphocytes from asthmatic patients

Background: L-selectin (CD62L) mediates the binding of lymphocytes to high endothelial venules of peripheral lymph nodes and is also involved in leukocyte attachment to the endothelium at sites of inflammation. Although it has been demonstrated that L-selectin is shed after lymphocyte activation, it is unknown whether the expression of L-selectin on the surface of lymphocytes can be modulated by an IgE-dependent mechanism or whether immunotherapy (IT) might affect this mechanism.

Methods: One group of adult allergic asthmatic patients had received IT for the previous 3 years. Another similar group was not treated with IT. We challenged peripheral blood lymphocytes from both groups of asthmatic patients *in vitro* with an anti-IgE antibody (Ab). Expression of L-selectin on the lymphocyte surface was analyzed by flow cytometry, and the levels of soluble L-selectin (sL-selectin) on culture supernatant by ELISA.

Results: L-selectin was downregulated from the surface of lymphocytes in a time- and anti-IgE antibody dose-dependent manner (with a concomitant upregulation of shed L-selectin in the supernatant). When lymphocytes from non-IT asthmatic patients were cultivated with anti-IgE Ab, a statistically significantly greater CD62L downmodulation on the lymphocyte surface was observed compared with lymphocytes from the healthy group ($P < 0.002$) and from the IT-asthmatic group ($P < 0.001$). When lymphocytes from non-IT asthmatic patients were cultivated with anti-IgE Ab, a significantly greater sL-selectin level in the culture supernatant was observed compared with lymphocytes from the healthy group ($P < 0.001$) and with lymphocytes from IT-asthmatic group ($P < 0.001$).

Conclusions: We present evidence that the expression of L-selectin on the surface of lymphocytes can be modulated by an IgE-dependent mechanism. This mechanism can be affected by IT.

**J. Monteseirín¹, E. Llamas¹,
H. Sánchez-Monteseirín¹,
I. Bonilla¹, M. J. Camacho¹,
J. Conde¹, F. Sobrino^{1,2}**

¹Departamento de Medicina, Servicio Regional de Inmunología y Alergia, Hospital Universitario Virgen Macarena; ²Departamento de Bioquímica Médica, Facultad de Medicina, Universidad de Sevilla, Seville, Spain

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Dr J. Monteseirín
Asunción 27
41011 Sevilla
Spain

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The localization of leukocytes at vascular sites of inflammation involves several discrete processes including cell capture, rolling, activation, and arrest (1). After arrest, many leukocytes migrate between endothelial junctions into the perivascular tissue. At least three families of adhesion receptors mediate this coordinated sequence of events: selectins, integrins, and the Ig gene superfamily. Selectins, consisting of L-selectin (CD62L) on leukocytes and E- (CD62E) and P-selectin (CD62P) on endothelial cells, have been shown to mediate capture and rolling (1), but not to mediate cell arrest under conditions of flow (2). L-selectin is normally expressed on the surface of most leukocytes, including lymphocytes, neutrophils, monocytes, eosinophils, hematopoietic progenitor cells, and immature thymocytes (3, 4). L-selectin mediates the binding of lymphocytes to high endothelial venules of peripheral lymph nodes (3), and is also involved in leukocyte attachment to endothelium at sites of inflammation (5). Leukocytes from mice lacking

L-selectin are deficient in both activities (6). Human L-selectin is a highly glycosylated protein of 95 000–105 000 M_r on neutrophils and 74 000 on lymphocytes (4). L-selectin is shed from the cell surface after cellular activation *in vitro* after exposure to the nonphysiologic stimulator phorbol esters (PMA) (7), B-cell growth factors and Ig cross-linking (8), IL-2 (9), an anti-CD3 monoclonal antibody (mAb) (10), extracellular ATP (11), mitogens (PWM, Con A, and PHA) (12), an anti-Leu 13 mAb, and the mAb DREG-56 against the extracellular domain of L-selectin (13). It has been proposed that endoproteolytic release of L-selectin might provide a rapid means for the regulation of leukocyte deadhesion necessary to enable leukocytes to detach themselves from bound endothelial cells before transmigration between endothelial cells and into sites of inflammation (14). However, the protease(s) mediating endoproteolytic release of L-selectin appear(s) to be ubiquitous, as opposed to cell type-specific, since a

broad array of L-selectin-negative cell types transfected with L-selectin cDNA are able to shed this receptor (15, 16). Shed L-selectin (sL-selectin) is found in normal plasma at high levels ($\approx 1\text{--}3\text{ }\mu\text{g/ml}$) and retains functional activity (15). Thus, not only may the endoproteolytic release of L-selectin be a means of regulating cell-surface receptor expression, but also the shed receptor may modulate leukocyte binding to endothelium *in vivo*. Despite this evidence, it is not known whether the expression of L-selectin on the surface of lymphocytes can be modulated by an IgE-dependent mechanism. The present work was undertaken to analyze this problem. Here, we present evidence that an anti-IgE antibody (Ab) was able to regulate expression of L-selectin on lymphocytes from allergic patients.

Material and methods

Materials

Goat anti-human IgE antibody was obtained from Caltag Laboratories (Barcelona, Spain). Other biochemicals were obtained from Sigma (Madrid, Spain), Serva (Barcelona, Spain), and Merck (Barcelona, Spain).

Patients and controls

The studied groups included adult atopic patients with bronchial asthma and healthy adult nonatopic volunteer controls. One group of asthmatic patients ($n=15$) had positive skin prick test (Bial-Aristegui, Bilbao, Spain) and specific IgE (HYTEC 288, Hycor Biomedical, Inc., Irvine, CA, USA) to *Dermatophagoides pteronyssinus*, and two patients also had positive skin prick test and specific IgE to grass pollen (*Phleum pratense*, *Lolium perenne*, *Poa pratensis*, and *Dactylis glomerata*). The subjects received no specific hypo-sensitization (non-immunotherapy [IT] asthmatic group). Another group of asthmatic patients ($n=15$) had received IT with *D. pteronyssinus* extract (Bial-Aristegui, Bilbao, Spain) for the previous 3 years and continued to receive a maintenance dose within the highest potency of the extract (IT-asthmatic group). The patients suffering intermittent asthma (17) were not allowed to take any bronchodilators within 8 h before challenge of lymphocytes *in vitro*. Oral bronchodilators were withheld for 24 h, and none of the subjects had taken corticosteroids, disodium cromoglycate, or nedocromil sodium in the previous week. The healthy controls ($n=15$) had no history of allergy or bronchial symptoms, and had negative skin prick test (Bial-Aristegui) and specific IgE (HYTEC 288) to a battery of inhalant allergens (house-dust mites, pollens, molds, and animal danders). The study was approved by the Hospital Research Committee of the Department of Medicine, and each subject gave informed consent.

Preparation of peripheral blood lymphocytes

Peripheral blood lymphocytes were obtained from venous blood samples by standard gradient separation techniques (18). Venous blood (20 ml) from patients was added to heparin and diluted with an equal volume of Hepes-buffered NaCl medium (145 mM NaCl, 5 mM KCl, 10 mM Hepes, pH 7.4, 5 mM D-glucose, and 1 g/l bovine serum albumin) supplemented with 1 mM CaCl_2 . Mononuclear cells were separated by density gradient centrifugation over Ficoll-Paque, washed twice, and resuspended in Hepes-buffered NaCl medium.

Lymphocyte stimulation with anti-IgE Ab

Cells, resuspended at 1×10^6 in Hepes-buffered NaCl medium, were stimulated with the anti-IgE Ab at 37°C at the time, and the concentrations are expressed in Results and the legends of figures.

Flow cytometry analysis

After each experiment, cells were rapidly cooled on ice, and 1 ml of cold wash buffer (PBS) was added. Cells were then centrifuged at 500 g for 10 min, supernatants were removed, and cell pellets were washed three times with cold wash buffer before staining with 20 μl of PE-labeled CD62L or FITC-labeled CD4, CD8, and CD20, along with control cells stained with PE- or FITC-labeled IgG2b-nonspecific control Ab (Coulter Corp., Hialeah, FL, USA). Flow cytometry was performed on an Epics Elite flow cytometer (Coulter Corp., Hialeah, FL, USA) and calibrated by standard techniques. For each stained sample, 2×10^4 cells were analyzed for fluorescence intensity. The fluorescent distribution for both control and test cells was analyzed with a Coulter Elite software workstation. Results are expressed as frequency of fluorescence-positive cells (%) (19).

Analysis of shed L-selectin (sL-selectin) by ELISA

sL-selectin levels in culture supernatants were determined in replicate wells by sandwich ELISA (Bender Med Systems, Barcelona, Spain). Samples were diluted 1:100 before analysis. The limit of sensitivity determined by the manufacturer was 0.3 ng/ml.

Statistics

Data are expressed as median and 25th, and 75th percentiles. Comparisons between groups were made by the Mann-Whitney U-test. A P value of <0.05 was considered significant.

Results

Fig. 1 illustrates the dose-dependent response in the expression of CD62-L when cells (from the non-IT asthmatic group) were challenged with different doses of anti-IgE Ab. At 18 h, increasing concentrations of anti-IgE Ab induced a progressively greater loss of L-selectin,

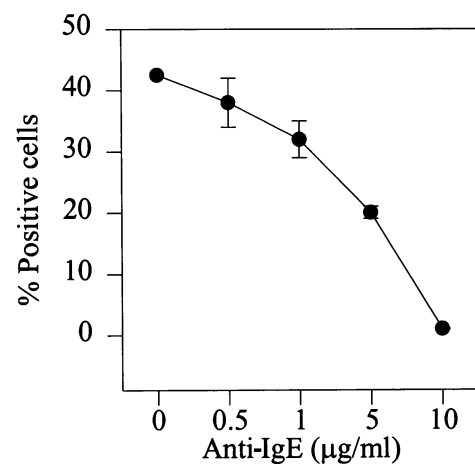


Figure 1. Dose-dependent expression of CD62L when cells from non-IT asthmatic group were challenged with different doses of anti-IgE Ab. At 18 h, increasing concentrations of anti-IgE Ab induced progressively greater loss of L-selectin, which was maximum at 10 $\mu\text{g/ml}$.

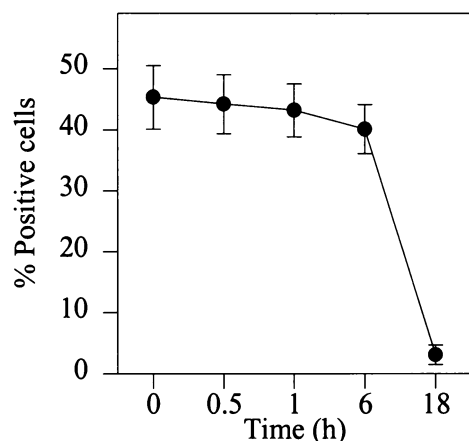


Figure 2. Kinetics of downregulation of CD62L on lymphocytes from non-IT asthmatic group, challenged with dose of anti-IgE Ab of 10 μ g/ml, over 18-h period.

which was maximum at 10 μ g/ml. The present data show that anti-IgE Ab also triggered significant downregulation of L-selectin on peripheral blood lymphocytes, although with delayed kinetics. In this regard, reduction of L-selectin expression by lymphocytes (from the non-IT asthmatic group) occurred during continuous exposure to anti-IgE Ab (10 μ g/ml) over an 18-h period, so that only a small proportion of cells expressed L-selectin at a low density (Fig. 2).

When lymphocytes from non-IT asthmatic patients were cultivated with anti-IgE Ab (10 μ g/ml at 18 h), a statistically significantly greater CD62L downmodulation on the lymphocyte surface was observed compared with lymphocytes from the healthy group ($P < 0.002$) and from the IT-asthmatic group ($P < 0.001$) (Fig. 3A). In the non-IT asthmatic group, after 18 h of incubation with anti-IgE Ab (10 μ g/ml), the downmodulation of CD62L in lymphocyte subsets CD4, CD8, and CD20 was similarly affected, as indicated by two-color labeling experiments (data not shown).

L-selectin shedding is known to be a major mechanism by which L-selectin density on the plasma membrane is regulated after activation of lymphocytes. Therefore, to determine whether anti-IgE Ab initiates L-selectin downmodulation from lymphocyte cell surfaces through a shedding mechanism, soluble L-selectin levels in culture supernatants were determined by ELISA. When lymphocytes from non-IT asthmatic patients were cultivated with anti-IgE Ab (10 μ g/ml at 18 h), a significantly greater sL-selectin level in the culture supernatant was observed compared with lymphocytes from the healthy group ($P < 0.001$) and with lymphocytes from the IT-asthmatic group ($P < 0.001$) (Fig. 3B).

Discussion

Extravasation and activation of lymphocytes are essential in the inflammatory response. Leukocyte extravasa-

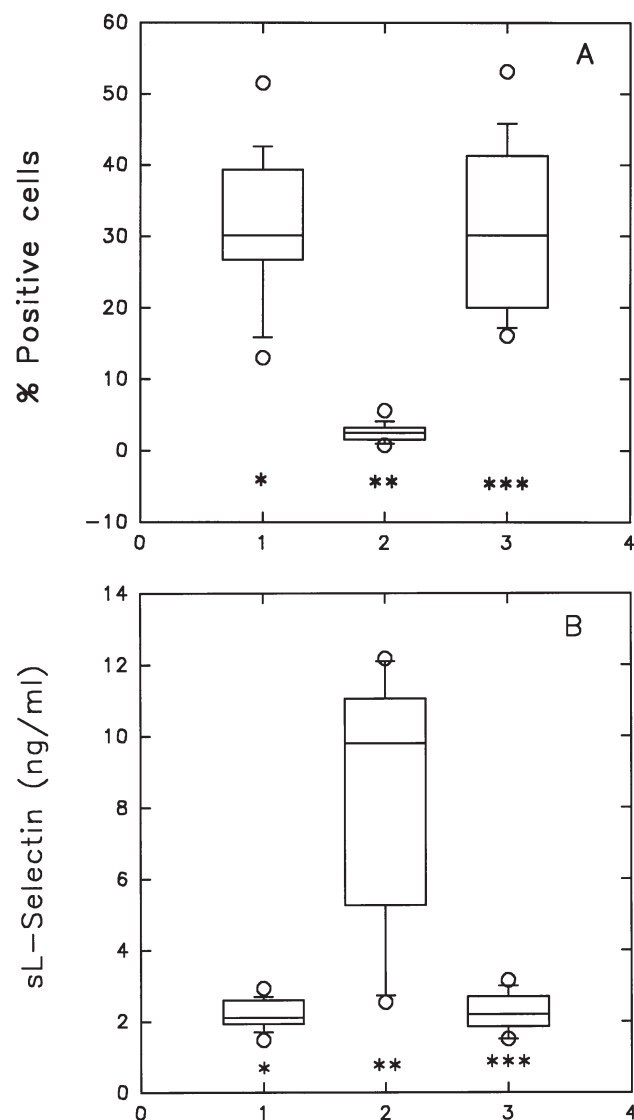


Figure 3. A) When lymphocytes from non-IT asthmatic patients (**) were cultivated with anti-IgE Ab (10 μ g/ml at 18 h), significantly greater CD62L downmodulation on lymphocyte surface was observed compared to lymphocytes from healthy group (*) ($P < 0.002$) and from IT-asthmatic group (***) ($P < 0.001$). B) Soluble L-selectin levels in culture supernatants were determined by ELISA. When lymphocytes from non-IT asthmatic patients (**) were cultivated with anti-IgE Ab (10 μ g/ml at 18 h), significantly greater sL-selectin level in culture supernatant was observed compared to lymphocytes from healthy group (*) ($P < 0.001$) and with lymphocytes from IT-asthmatic group (***) ($P < 0.001$).

tion into inflamed areas involves a complex interaction of leukocytes with endothelium through regulated expression of surface adhesion molecules. Of these, L-selectin appears to play a key role in the initial attachment of circulating leukocytes to endothelium. The experiments described here provide the first evidence of a novel level of regulation of L-selectin expression that involves the IgE signal. Specifically, engagement with anti-IgE Ab was shown to result in a marked inhibition of L-selectin

surface density on peripheral blood lymphocytes. Moreover, loss of L-selectin surface expression on lymphocytes was attributed to an increase in L-selectin shedding from the plasma membrane.

The physiologic relevance of the present data is stressed by the fact that lymphocytes are present in the sites of allergic inflammation (20, 21). The chronic airway inflammation found in asthma is characterized by a strikingly selective accumulation of eosinophils and lymphocytes (22). *In vitro* studies conducted to determine the relationship between the transendothelial migratory capacity of normal peripheral lymphocytes and their surface phenotype have demonstrated that a subset of CD62L-negative memory cells exhibits this capacity (23). After challenge of 10 atopic patients with allergen and monitoring of the cellular influx for 12 h with a skin blister chamber model, T cells had reduced levels of L-selectin compared with the same cells in blood (24).

Thus, an IgE-dependent mechanism might lead to direct lymphocyte extravasation into allergic sites. It is presumed that the shedding of L-selectin is a prerequisite for cell emigration and permits leukocyte extravasation (24). Furthermore, sheep treated with aerosol of an L-selectin-specific mAb before bronchial antigen challenge had a significantly reduced late airway response and did not develop postchallenge airway hyperresponsiveness. No protective effect was seen when sheep were treated with a nonspecific control mAb. Treatment with the L-selectin-specific mAb also reduced the severity of the early airway response to antigen. Thus, L-selectin may have a cellular function that modulates allergen-induced pulmonary responses (25). On the other hand, the majority of helper cells that produce IL-4 express an L-selectin^{low} phenotype, consistent with their existence as memory or effector populations. The IL-4 produced by these cells is thought to promote the generation of IL-4-producing cells from naive, L-selectin^{high} CD4⁺ T cells (26). These data support the idea that T cells with L-selectin phenotypes contain Th2-like cytokine-producing cells, and are in concordance with the fact that asthmatic inflammation is driven, at least in part, by clonally expanded Th2 cells (27).

L-selectin is rapidly shed after leukocyte activation (2). When we stimulated the lymphocytes of allergic

patients with an anti-IgE Ab, the downregulation of CD62L determined an increase of sCD62L in the supernatant of lymphocytes in agreement with other activating factors such as PMA, ATP, PWM, Con A, and PHA that affect cell-surface L-selectin expression (15). In the same experiments, when we stimulated lymphocytes with anti-IgE Ab, the cell surface downmodulation of L-selectin directly correlated with an increase of sL-selectin in culture supernatant fluid. Although the functional significance of sL-selectin is still unknown, serum levels of sL-selectin in patients with atopic dermatitis were significantly higher than those found in normal control subjects. Furthermore, sL-selectin levels correlated positively with disease severity and total serum IgE levels in atopic dermatitis (28). On the other hand, in children with stable asthma, the mean serum of sL-selectin was significantly higher than that in controls (29). The level of sL-selectin may be a useful immunologic indicator for disease activity in atopic processes.

We present evidence that allergen-IT of individuals suffering from allergic asthma was associated with a significant reduction in the downregulation of CD62L on the surface of lymphocytes and in the levels of sL-selectin in the culture supernatants, from these patients when the cells were challenged *in vitro* with anti-IgE Ab, in comparison with the cells of asthmatic patients without IT. It could be hypothesized that the action of IT in L-selectin expression on lymphocyte might inhibit adhesion of these cells to the endothelium and consequently lymphocyte migration into allergic sites.

In conclusion, we present evidence that the expression of L-selectin on the surface of lymphocytes can be modulated by an IgE-dependent mechanism. This mechanism can be affected by IT.

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