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Peripheral Blood Lymphocyte Activation and RANTES Levels in Asthma

Gaye ERTEN[1]
Benan MÜSELLİM[2]
Bülent TUTLUOĞLU[2]
Bilun GEMİCİOĞLU[2]
Günnur DENİZ[1]

[1] Department of Immunology, Institute of Experimental Medicine, İstanbul University, İstanbul - TURKEY

[2] Department of Pulmonary Diseases, Cerrahpaşa Faculty of Medicine, İstanbul University, İstanbul - TURKEY

Aim: Bronchial mucosal inflammation is one of the major characteristics of atopic asthma. Th2 activation and the related cytokine profile, eosinophil activation and infiltration play the main role in the pathogenesis of atopic asthma. The aim of this study was to demonstrate the activation and RANTES (regulated on activation, normal T-cell expressed and presumably secreted) expression of peripheral blood lymphocytes of non-atopic and atopic asthmatic patients.

Materials and Methods: CD3, CD4, CD8, CD16, CD23, CD25, CD45RA and CD45RO expressions were determined in 22 asthma patients and 20 healthy control subjects by flow cytometry, and RANTES levels were measured by ELISA. Statistical analysis was performed by using Student's t and Mann-Whitney U test.

Results: CD45RO and CD23 expressions were significantly higher in asthma patients compared to control subjects (P = 0.009 and P = 0.004, respectively), and similarly, an increase in CD25 expression was also shown in asthmatics (P = 0.004). However, there was no difference in RANTES secretion of peripheral blood lymphocytes in asthmatics compared to the control group (P = 0.08). Atopic and non-atopic asthmatics (13 vs. 9) were compared, and atopic asthmatics showed significant increase in CD25 and CD23 expressions (P = 0.009 and P = 0.02, respectively).

Conclusions: These changes in the activation state of T-cells suggest an active role of T lymphocytes in the pathogenesis of atopic and non-atopic asthma.

Key Words: Asthma, lymphocyte activation, RANTES

Astımda Periferik Lenfosit Aktivasyonu ve RANTES Düzeyleri

Amaç: Bronşiyal mukozal inflamasyon atopik astımın en temel özelliklerinden biridir. Th2 aktivasyonu ve buna bağlı sitokin profili, eozinofil aktivasyonu ve infiltrasyonu atopik astımın patogenezinde önemli rol almaktadır. Bu çalışmada amaç non-atopik ve atopik astmatik olgularda periferik kan lenfositlerinde aktivasyonu ve RANTES (normal T hücrede eksprese ve sekrete edilen, aktivasyonla düzenlenen) ekspresyonunu ortaya koymaktır.

Yöntem ve Gereç: CD3, CD4, CD8, CD16, CD23, CD25, CD45RA ve CD45RO ekspresyonları 22 astım hastası ve 20 sağlıklı kontrol olgusunda flow sitometri ile; RANTES düzeyleri ise ELISA ile saptanmıştır. İstatistiksel analizde Student's t testi ve Mann Whitney U testi kullanılmıştır.

Bulgular: CD45RO ve CD23 ekspresyonları astımlı olgularda sağlıklı bireylere göre anlamlı olarak yüksek bulunmuştur (P = 0.009 ve P = 0.004, sırasıyla), benzer şekilde CD25 ekspresyonu da astımda yükselmiştir (P = 0.004). Buna karşılık periferik lenfosit RANTES salınımında astmatik olgular ve sağlıklı bireyler arasında fark saptanmamıştır (P = 0.08). Atopik ve non-atopik astımlı hastalar (13/9) karşılaştırıldığında; atopik astımda CD25 ve CD23 ekspresyonları anlamlı düzeyde yüksek bulunmuştur (P = 0.009 ve P = 0.02, sırasıyla).

Sonuç: T hücrelerinin aktivasyon durumundaki değişiklikler bu hücrelerin atopik ve non-atopik astım patogenezindeki rolünü işaret etmektedir.

Anahtar Sözcükler: Astım, lenfosit aktivasyonu, RANTES

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Correspondence
Gaye ERTEN
Department of Immunology, Institute of Experimental Medicine, İstanbul University, İstanbul - TURKEY
Vakif Gureba Caddesi 34393 Şehremini, İstanbul - TURKEY

gerten@istanbul.edu.tr

Introduction

Asthma is a chronic inflammatory disease characterized by increased responsiveness of the tracheobronchial tree to a variety of stimuli. Clinically, asthma can be subdivided into the variants as extrinsic and intrinsic asthma. Extrinsic asthma is also known as allergic, or atopic, and generally develops in childhood, and family history is common. Intrinsic asthma, however, known as nonallergic or non-atopic, develops after upper respiratory tract infections, occurs unrelated to pollen seasons and skin tests are negative

(1). In atopic asthma, allergen-specific IgE bound to mast cell via Fc receptor plays a role in the activation of the mast cell causing the release of the granule contents (histamine, tumor necrosis factor [TNF]-α, proteases), synthesis of a variety of lipid membrane-derived molecules (heparin, LTC4, LTB4, PGD2, PAF) and the production of a number of cytokines (interleukin [IL]-1, IL-3, IL-4, IL-5, IL-6, IL-8, TNF-α granulocyte- macrophage colony-stimulating factor [GM-CSF] and macrophage inflammatory protein [MIP]-1α) (2). The result is vasodilatation, increased vasopermeability and increased endothelial adhesiveness to leukocytes. The inflammatory cells, like eosinophils, basophils, macrophages and lymphocytes, reach the lung and release their own mediators causing bronchoconstriction, mast cell multiplication, mucus secretion and tissue damage. Th2 lymphocyte activation has been shown to play the key role in the inflammatory response in asthmatic airways. This can be shown by increased numbers of CD25 (IL-2Rα) of T-cells and by IL-4-mediated B-cell expression of CD23 (IgE receptor low affinity) (3). The Th2 hypothesis of allergy considers atopy as a Th2-driven hypersensitivity reaction driven by IL-4, IL-5 and IL-13 (4). Chemokines stimulating chemokinesis and chemotaxis also play a role in allergy and asthma. Chemokines of the beta subfamily do stimulate human eosinophils and basophils and are considered to be mediators of inflammation. RANTES (regulated on activation, normal T-cell expressed and presumably secreted), a CC chemokine, can be released from activated macrophages and is a potent chemoattractant for eosinophils, monocytes and T-cells (5), and acts in acute inflammatory responses (6). A report by the Manchester group showed an interesting link between RANTES polymorphisms and asthma. Their report shows that the rare variant of the RANTES gene is associated with high risk of asthma and severe airway obstruction (7). In this study, lymphocyte activation and related RANTES levels were investigated in atopic and non-atopic asthma.

Materials and Methods

Study Population

Twenty-two asthmatic patients (mean age 36 ± 8) and 20 healthy control subjects (mean age 38 ± 12) were enrolled in this study (8). Characteristics of the patients are shown in Table . All the patients participating in this study were mild non-smoker asthmatics under inhaled corticosteroid (ICS) ± long-acting beta agonist (LABA) therapy. Their asthma symptoms had been under control for at least one month.

The study protocol was approved by the Ethical Board of the Istanbul Faculty of Medicine.

Cell Purification

Peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood by density gradient centrifugation over Ficoll (Sigma Chemical Company, St. Louis, MO, USA), and washed twice by phosphate buffered saline (PBS) (pH 7.4). The cells were stained with PE- or FITC-conjugated isotype control antibodies (Abs) (rat IgG1 and rat IgG2a), anti-human CD4-FITC/CD8-PE (Caltag, Burlingame, CA, USA), anti-human CD3-FITC/CD19-PE (Ancell, Bayport, MN, USA), anti-human CD16-FITC (DAKO, Carpinteria, CA, USA), anti-human CD45RO-PE (Pharmingen, San Diego, CA, USA), anti-human CD45RA-FITC (Pharmingen, San Diego, CA, USA), anti-human CD25-FITC (Pharmingen, San Diego, CA, USA), anti-human CD23-PE (Pharmingen, San Diego, CA, USA) for 30 min at 4°C and analyzed by flow cytometry (Epics Profile II, Coulter, USA).

Quantitation of RANTES

The solid phase sandwich ELISA for RANTES was performed by ELISA (Endogen, Cambridge, MA, USA). The sensitivity of RANTES ELISA was <2 pg/ml.

Statistical Evaluation

Data are expressed as means ± SEM. Statistical analyses for comparisons were performed by Student's *t* and Mann-Whitney U test.

Table. Characteristics of the asthmatic subjects.

	Atopic	Non-atopic	Control
Number of subjects	13	9	20
Sex (male/female)	4/9	2/7	10/10
Mean age	35.2 ± 12.8	38.8 ± 12.7	36.3 ± 8.5
Asthma duration (year)	9.6 ± 4.1	10.3 ± 5.2	-
ICS usage (N)	13	9	-
LABA usage (N)	3	2	-
FEV1 (% PRED)	87 ± 13.6	86 ± 18.5	92.2 ± 8.4

ICS: inhaled corticosteroid; LABA: long-acting beta agonists; FEV1: forced expiratory volume in one second.

Results

Antigen Expression

Surface expressions of the low affinity IgE receptor CD23 and memory T-cell marker CD45RO were increased in the peripheral blood of all asthmatics compared to healthy subjects (P = 0.004 and P = 0.009, respectively, Figure 1), but there was no difference in CD3 expressed on T-cells or in CD4 on class II MHC restricted T-cells in asthmatic and control groups (data not shown). Expressions of CD8, MHC class I restricted T-cell marker; CD16, NK marker; CD19, expressed on most B–cells; and CD45RA, expressed on naive T–cells, did not show any difference between asthmatic and healthy individuals (data not shown). However, an increase was determined in IL-2R expressed on activated T- and B-cells, marked as CD25, in the asthmatic group compared to healthy subjects (P = 0.004, Figure 1). When differentiating the asthmatic group into skin test-positive (n:13) and -negative (n:9) subjects (to divide the asthmatic group into atopic and non-atopic), skin test-positive asthmatics showed a significant increase in CD25 and CD23 expression compared to skin test-negative subjects (P = 0.009 and P = 0.02, respectively, Figure 2). CD4/CD8 ratios did not show any difference in control and asthmatic groups (2.00 ± 0.89 vs 1.90 ± 0.21).

RANTES Levels

Although an increase was shown in chemokine RANTES determined by ELISA in the asthmatic group compared to healthy individuals, this increase was not statistically significant. RANTES levels of skin test-positive and skin test-negative asthmatic patients also did not show any difference (812.42 ± 219.89 pg/ml vs 851.33 ± 292.95 pg/ml) (Figure 3).

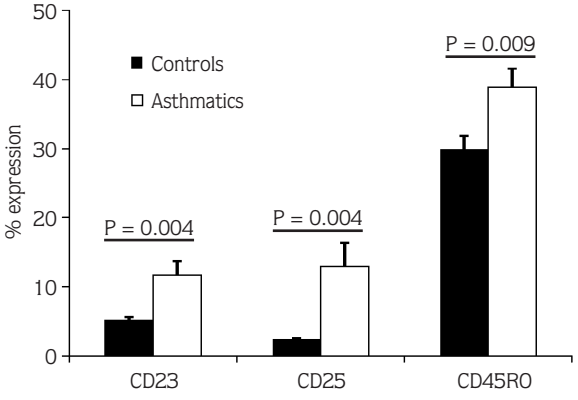

Figure 1. Expression of CD23, CD25 and CD45RO in asthmatic and control subjects.

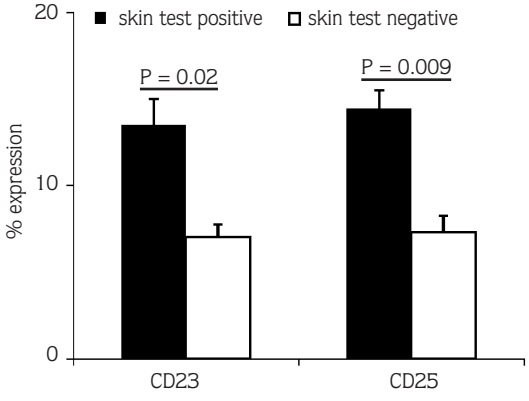

Figure 2. CD23, CD25 expressions in skin test-positive (atopic) and -negative (non-atopic) asthmatic subjects.

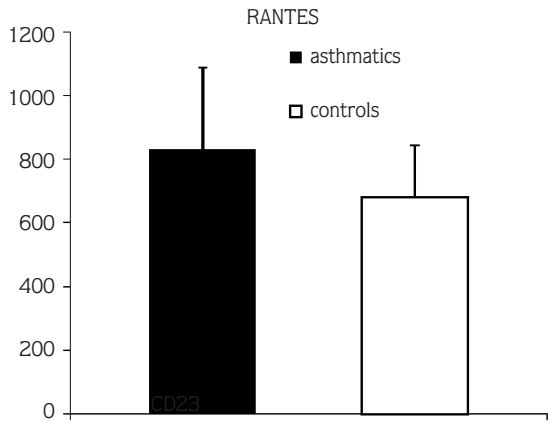

Figure 3. Serum RANTES levels in asthmatic and control subjects.

Discussion

The role of inflammation in the pathogenesis of asthma has been well described (9). Initial studies of circulating T lymphocytes have suggested an increased activation of these cells during exacerbations of asthma (10). Extending the investigation of cell activity to the airways, a study comparing T lymphocyte cell surface activation markers, IL-2 receptor (IL-2R, $CD25^+$) and the class II major histocompatibility antigen, HLA-DR, in peripheral blood and bronchoalveolar lavage (BAL) in mild to moderately severe asthmatics and healthy control subjects has shown a small but significant increase in the state of activation of $CD3^+$ T lymphocytes in asthma, which was restricted to BAL cells (11). The lack of a significant difference in CD3 levels in asthmatics compared to controls in this study was thought to be due to the patients being mild asthmatics.

The large majority of lymphocytes in the airway mucosa are T-cells, which are likely to play a central role in regulating inflammatory responses in the airways. Examination of T-cell subsets has generally demonstrated a predominance of CD4± cells over CD8± (suppressor/cytotoxic) cells (12). Following antigen challenge, changes in the T-cell subpopulation in the airway sampled by BAL occur as early as 10 minutes after instillation. Gratziou et al. (13) demonstrated lost lymphocytes from the airway of asthmatics 10 minutes following segmental challenge. These lost cells were predominantly of the CD4 phenotype but fewer CD8 cells were recovered as well. Moreover CD4/CD8 ratio fell significantly over this short time period. Marked variability in the CD4:CD8 ratio occurs; however, no consistent alterations in the ratio of these T-cell subsets have been reported in asthma (12). In contrast, no significant difference in peripheral blood CD4/CD8 levels was found. CD23 has been found at increased levels in asthmatic and atopic individuals in previous studies and has been implicated in other diseases characterized by chronic inflammation (14,15). Similar to these findings, our results showed significantly high CD23 expression in asthmatics compared to healthy individuals and atopic asthmatics compared to non-atopics. Several functional roles for CD23 have been described, including T-cell-dependent IgE production, augmentation of B-cell proliferation, enhancement of antigen presentation, and mediation of T- and B-cell cognate interactions (16). CD23 has also been linked to receptor-ligand interactions between T- and B-cells with effects on the CD40-CD40L pathway and the β2-integrins (17). It is believed that CD23 negatively regulates pulmonary inflammation and airway hyperreactivity (18).

Another parameter for evaluating Th2 lymphocyte activation is determination of CD25 expression of T helper cells. A study performed by Hallstrand and coworkers (3), demonstrated that the number of CD23-bearing B-cells and the number of CD25-bearing T-cells were increased in asthma compared to controls. With the development of exercise-induced bronchospasm, there was a significantly greater increase in CD23-positive B-cells and CD25-positive B-cells in the asthma group. Similarly higher expressions of C23 and CD25 were also found in asthmatic patients.

The CD45 surface marker is found on all leukocytes. Several structural variants of this molecule have been described (19). Consistent with T-cell populations in other mucosal tissues, most T-cells in the airways carry CD45RO surface marker associated with memory T-cells. This surface phenotype identifies T-cells that have undergone prior activation by antigen (12). Several studies have reported increased T-cells, number of lymphocytes, or $CD45RO^+$ cells in asthmatics compared to healthy controls, but other studies have failed to demonstrate any increase (20) in CD25 expression (identifying IL-2R) on T-cells compared to normal and atopic, non-atopic controls. Increased CD25 expression was associated with EG2 (a secreted form of eosinophil cationic protein [ECP]) staining of eosinophils, implying a role of T-cells in the eosinophilic infiltrate in asthma (21). Eosinophils extravasating into inflamed tissues respond to chemotactic gradients and migrate toward epithelial cells. Production of chemokines by epithelial cells attracts critical inflammatory effector cells. RANTES is one of these chemokines that cause eosinophil chemotaxis (22). RANTES might be released from various cells including lymphocytes and activated platelets, and is considered to play an important role in immune and allergic disorders. Recent studies demonstrated the beneficial effects of topical steroids on airway inflammation in asthma by reducing RANTES-induced leukocyte infiltration into the airway wall (23). The findings suggest that certain antihistamines may act as down-modulators of allergic inflammation, possibly through a negative regulation of the chemokines involved in activation and attraction of eosinophils (24).

Many studies have also made an interesting link between RANTES polymorphisms and asthma, showing that the variant in the promoter region is associated with high risk of asthma and severe airway obstruction (25). In the patients with asthma, the plasma RANTES levels were found to be elevated during acute attack and the stable period compared to controls (26). In the present study, although higher levels of RANTES were found in asthmatics compared to controls, the difference did not reach statistical significance. That finding may be the result of ICS usage, which was shown to repress RANTES by inhibition of NF-kappaB-dependent transcription (27).

In conclusion, these data suggest that activation of T lymphocytes may be an event in the development of airway inflammation. Although this T-cell-mediated inflammation is more prominent in atopic asthmatics, non-atopic asthmatics also show signs of inflammation in the peripheral blood. RANTES secretion was not associated with this T-cell activation in our patients with mild stable asthma.

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