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Are thermogenic proteins and adipokine chemerin affected by monoclonal antibody therapy in asthma?

Tangul Bulut, Arzu Didem Yalcin[1,2], Betul Celik, Gizem Esra Genc[3], Ayse Yesim Gocmen[4], Saadet Gumuslu[3]

ORCID:
Tangul Bulut: https://orcid.org/0000-0003-2844-7819
Arzu Didem Yalcin: https://orcid.org/0000-0002-0615-5279
Betul Celik: https://orcid.org/0000-0001-9731-9939
Gizem Esra Genc: https://orcid.org/0000-0003-3113-985X
Ayse Yesim Gocmen: https://orcid.org/0000-0002-4036-5640
Saadet Gumuslu: https://orcid.org/0000-0002-1021-3662

Department of Pathology, Antalya Education and Research Hospital, Antalya, Turkey, [1]Department of Internal Medicine, Allergy and Clinical Immunology, Academia Sinica, Genomics Research Center, Taipei, Taiwan, [2]Department of Internal Medicine, Allergy and Clinical Immunology, Antalya Education and Research Hospital, [3]Department of Medical Biochemistry, Faculty of Medicine, Akdeniz University, Antalya, [4]Department of Medical Biochemistry, Faculty of Medicine, Bozok University, Yozgat, Turkey

Address for correspondence:
Prof. Arzu Didem Yalcin, Internal Medicine, Allergy and Clinical Immunology, Academia Sinica, Genomics Research Center, 11529 Taipei, Taiwan.
Internal Medicine, Allergy and Clinical Immunology, Antalya Education and Research Hospital, Antalya, Turkey.
E-mail: adidyal@yahoo.com

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Abstract:

BACKGROUND: Irisin is a thermogenic protein that sources outgoing energy by converting white adipose tissue to brown adipose tissue. Chemerin is originally identified as a chemoattractant protein that mainly mediating the chemotaxis of dendritic cells and natural killer cells (NKCs). The aim of this study is to assess the potential impact of immune modulation-related chemerin and irisin concentrations together with cell surface markers (CSM) in allergic asthmatic patients under omalizumab treatment.

MATERIALS AND METHODS: The study participants were age- and sex-matched 30 healthy controls (Group I) and consecutive patients who had severe persistent asthma disease (Group II). Asthma patients took omalizumab treatment for 12 months within every 2 weeks. Flow cytometry analysis was used to evaluate CSM, enzyme-linked immunosorbent assay (ELISA) for interleukin-1 (IL-1) β expression. In addition, NK activity (NKA) and induced cytokine expression (by bioassay and ELISA, respectively) before and after omalizumab therapy were evaluated.

RESULTS: Chemerin, irisin, and IL-1 β concentrations were significantly higher in severe persistent asthma patients compared to controls in serum ($P = 0.01$; $P = 0.03$; and $P = 0.008$, respectively). IL-1 β level decreased with treatment and it was statistically significant. Although levels decreased, no statistically significant difference was observed for Irisin, CD80, and CD56/16 levels. Chemerin level kept rising after treatment, and this was significant statistically.

CONCLUSIONS: This is the first study to assess NKA and adipokines in asthma patients and their relationship with CSM. We observed that the level of these molecules is higher in asthma and is influenced by omalizumab treatment. Since no obvious change was observed for NKCs, omalizumab may be considered safe against cancer development.

Keywords:

Asthma, CD16-56, CD80, chemerin, interleukin-1 β, irisin, natural killer activity, omalizumab

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Introduction

White adipose tissue synthesizes and secretes many molecules such as irisin,[1] and acts as a major endocrine tissue. Irisin is a thermogenic protein that sources outgoing energy by converting white adipose tissue to brown adipose tissue.[2] Boström *et al*. reported that fibronectin type III domain-containing protein 5 (FNDC5) messenger RNA in the skeletal muscle of mice and humans increased after exercise. Furthermore, they suggested that irisin, which is cleaved from FNDC5 by an unknown protease, is an exercise protein that is secreted into the circulation.[3]

Chemerin is recognized as a chemoattractant protein that mainly mediates the chemotaxis of plasmacytoid dendritic cells (DCs) (CD80) and natural killer cells (NKC, CD16, and CD56).[4-6]

Asthma is severely dependent on a series of cell adhesion molecule-mediated interactions among the airway smooth muscle, vascular endothelium, and leukocytes, leading to elevated serum immunoglobulin E (IgE). Omalizumab, a humanized monoclonal antibody, can neutralize free IgE and inhibit the IgE-mediated allergic pathway without sensitizing mast cells and basophils. The clinical benefit of omalizumab has been established in several large clinical trials.[7-9]

The aim of this study is to investigate the potential impact of immune modulation-related molecules and cell levels, such as NKC, chemerin and irisin, together with cell surface markers (CSM) in allergic asthmatic patients under omalizumab treatment. There were no human studies about the effects of irisin and chemerin in asthma patients previously.

Materials and Methods

Study population

Twenty-nine consecutive patients, who have severe persistent asthma disease (n = 29, Group II, 9 males and 20 females) have no other autoimmune and/or chronic disorders, are above the age group of 18 years, and are managed by the Clinical Immunology and Allergy Unit, were recruited for the study. Patients with asthma who admitted to our clinic were residents in Antalya without any immigration status. Group II was divided into two subgroups as follows: Group IIA (pre-omalizumab), when they were first seen and diagnosed and Group IIB (postomalizumab) after 12 months of treatment during the disease remission. Age- and sex-matched controls (n = 30, Group I, 13 males and 17 females) were diagnosed to have no autoimmune disorder or allergic disease, had no history of atopy, cardiac, liver, renal and pulmonary diseases, and were attending the outpatient clinic of the same department.

Patients all underwent omalizumab (anti-IgE, Xolair) treatment every 2 weeks for 12 months. Blood samples from Group IIA and Group IIB were collected in April, May, and June in which pollination is at the highest level in the City of Antalya.

Treatment protocol and patient control

Patients were asked to maintain asthma diaries describing their asthma treatment, and recording the total monthly oral corticosteroid dose. Patients were also asked to come to the hospital in case of exacerbation and if possible to the outpatient center at our allergy service during business hours instead of the emergency room to keep the treatment under control. Best Standard Care, following the recommendations of the Global Initiative for Asthma, included inhaled fluticasone 500 mg bid, inhaled salmeterol 50 mg bid, and oral methylprednisolone. Before starting omalizumab treatment, patients underwent a trial period of at least 12 months. The protocol followed for decreasing oral steroid administration was as follows: if the patient remained stable at the end of 2 weeks, the daily dose was decreased by a further 2 mg for the following weeks. Steroid dose was then increased to the previous level, and the process was repeated.

Collection of blood samples and biochemical assays

Flow cytometric analyses were performed using Epics Altra, Beckman Coulter devices in the immunology, and flow cytometry unit. Flow cytometry analysis was used to evaluate CD16, CD56, CD80, and enzyme-linked immunosorbent assay (ELISA) was used for interleukin-1 (IL-1) β expression. In addition, NKC and induced cytokine expression before and after omalizumab therapy were evaluated using bioassay and ELISA, respectively.

The concentrations of chemerin in the serum were measured using commercially available ELISA kits (Human Chemerin ELISA kit, BioVendor-Laboratorni Medicina AS, Cat No. RD191136200R, Brno, Czech Republic). The concentrations of irisin in the serum were measured using commercially available ELISA kits (Human Irisin ELISA kit, BioVendor-Laboratorni Medicina AS, Cat No. RAG018R). The enzymatic reactions were quantified in an automatic microplate photometer, and the chemerin levels were expressed as ng/mL. The mean interassay coefficient of variation (CV) percent and intra-assay CV percent for chemerin were 8.9% and 5.4%, respectively.

IL-1 β levels were measured using an ELISA kit (Diaclone, Catalog No: 851.610.005). All assays were conducted

according to the manufacturer's instructions. The samples that showed higher concentrations were diluted and measured in duplicate. Pulmonary function tests with the measurement of fractional exhaled nitric acid (FE_{NO}) were performed on the same day. Clinical assessment and adverse effects were evaluated at each bimonthly patient visits, including vital signs, full physical examination, any allergy incident details, total and specific IgE levels with pulmonary function tests (forced expiratory volume in first second/forced vital capacity [FEV1/FVC] rates), FE_{NO} concentrations, and asthma control test (ACT; Quality Metric Incorp.). FE_{NO} was assessed under the ATS/ERS guidelines. Regular medications were recorded, together with asthma severity and control test based on symptom load and treatment intensity.[10]

Statistical analysis

Results were presented as mean ± standard error of the mean and mean ± standard deviation (SD) Comparison of parameters between the two groups was performed using Independent Samples *t*-test. The relationship between the variables was determined using the Pearson's correlation analysis. Statistical significance was defined as $P < 0.05$. Statistical analyses were performed using SPSS software version 18.0 (IBM Corporation, New York, USA).

Ethics statement

All studies were approved by the Ethics Committee. All studies were conformed to the ethical guidelines of the 2008 Helsinki declaration. All participants provided witnessed written informed consent prior to entering the study.

Results

The demographic characteristics, age, smoking status, asthma test scores, and medication usage of the patient and control groups are presented in Table 1. There was no significant difference between the groups in terms of age, body mass index, and smoking. We observed that the FE_{NO} concentration levels (mean ± SD) decreased in the follow-up period (Group-IIA: 50.53 ± 8.23 and Group-IIB: 38.24 ± 5.65). ACT scores ($P < 0.05$, $P = 0.03$), FEV_1 ($P < 0.05$, $P = 0.03$), and FVC ($P < 0.001$, $P = 0.0005$) significantly increased after omalizumab treatment; that is, a clinical improvement was observed.

Table 1: Clinical findings of the patients

Parameters	Group-I: Healthy volunteers (*n*=30)	Group-IIA: Before omalizumab (*n*=29)	Group-IIB: After omalizumab (*n*=29)	*P*
Age (years)	38.8±9.2	49.4±9.6	49.6±9.8	0.1
Weight (kg)	77.3±8.5	79.3±6.9	78.5±5.7	0.1
BMI (kg m^2)	29.2±6.6	33.9±8.9	32.5±7.0	0.3
Smoker (%)	40	29.4	29.4	0.8
FeNo (ppb)	-	50.5±8.2	38.2±5.6	0.0002
FEV1%	-	69.2±9.2	85.6±4.2	0.03
FVC %	-	68.5±5.4	87.4±5.9	0.0005
ACT score	-	14.8±3.2	21.4±1.8	0.03

Data are expressed as mean±SD. Group-I: Healthy volunteers, Group-IIA: Before omalizumab, Group-IIB: After omalizumab, FEV_1: Forced expiratory volume in 1 s, FVC: Forced vital capacity, FeNO: Fractional exhaled nitric oxide, ACT: Asthma control test (Quality Metric Incorp.), BMI: Body mass index, SD: Standard deviation

Table 2: Laboratory findings of the patients

Markers	Group-I: Healthy volunteers (*n*=30)	Group-IIA: Before omalizumab (*n*=29)	Group-IIB: After omalizumab (*n*=29)	*P*
Fasting glucose (mmol/l)	4.7±0.4	4.9±0.6	4.8±0.7	0.1
Fasting serum free fatty acids (mmol/l)	0.5±0.2	0.5±0.1	0.5±0.1	0.1
Fasting triglycerides (mmol/l)	1.4±0.3	1.4±0.3	1.4±0.4	0.3
Eosinophil (mm^3)	220.6±13.5	668.3±15.4	494.8±12.8	0.06
Total IgE (U/L)	65.4±10.7	425.5±10.6	408.9±8.2	0.03
IL-1β (pg/ml)	43±0.7	49.7±2.1	46.3±0.8	0.008
Irisin (μg/ml)	0.9±0.02	1.04±0.02	0.99±0.03	0.03
Chemerin (ng/mL)	146.1±0.8	155.8±3.3	163.11±3.2	0.01
Natural killer activation	39.4	41.8	42.9	0.6
Leucocyte (per cubic millimeter)	6220±2526	8150±2316	7950±1997	0.04
Hemoglobin (g/dL)	14.2±1.26	15.8±2.7	15.1±1.9	0.2
Flow cytometry				
CD16-56	150.2±1.6	152.5±1.03	154.8±0.7	0.3
CD80 (B7-1; BB1)	53.4±1.1	55.9±1.09	54.8±0.4	0.2

Data are expressed as mean±SEM. Group-I: Healthy volunteers, Group-IIA: Before omalizumab, Group-IIB: After omalizumab, IL: Interleukin, SEM: Standard error of the mean

The general characteristics and laboratory test results of the patients and controls are summarized in Table 2. There was no significant difference between the groups in terms of fasting glucose, fasting triglyceride, fasting serum free fatty acid, and hemoglobin levels as well as CSM NK activity (NKA), CD80, and CD56/16.

The total IgE, eosinophil count, FE_{NO}, and serum IL-1 β (pg/mL) levels significantly decreased after omalizumab treatment; that is, proinflammatory mediators decreased. Furthermore, we observed a decreased irisin level after treatment. When we compared severe persistent asthma patients to healthy volunteers, circulating chemerin, irisin, IL-1 β levels, and leukocyte count (per cubic millimeter, values represent mean ± SD) were significantly higher in severe persistent asthma patients. All data of the levels are presented in Table 2, Figures 1 and 2. Interestingly, chemerin level after omalizumab treatment continued to increase, which was statistically insignificant (P = 0.124) [Figure 1].

Pearson's correlation regression analysis was performed between the variables, but no significant data were found.

Discussion

Dysregulated expression of pro- and anti-inflammatory mechanisms is thought to be responsible for the development of chronic inflammation. Skeletal muscle dysfunction is a major complication of chronic obstructive pulmonary disease, especially in mild and advanced asthma diseases.[11] As a result of muscle weakness, reduced exercise performance occurs,[12] and mortality increases because of airflow obstruction.[13] Physical inactivity has been apparent in the muscles of the lower limb, especially the quadriceps, among asthma patients.[14,15] However, soluble exercise markers such as irisin have not been searched in these articles. In this study, we showed that circulating irisin levels were significantly higher in the severe persistent asthma patients compared with the healthy volunteers.

A novel myokine, irisin is localized in the skeletal muscle. The separation and secretion of irisin are similar to those of transmembrane polypeptides, such as epidermal growth factor and transforming growth factor (TGF) alpha.[3] In our previous article, regarding omalizumab and TGF, we observed the significant changes in the levels of the CXCL8, IL10, TGF-β, granulocyte colony-stimulating factor, and eosinophil cationic peptide prior to the start of omalizumab treatment and 12 months after its onset. It was confirmed to have a useful therapeutic effect,[16,17] and revealed the concept of how the major groups of cytokines could be used in asthma treatment.

Recent studies demonstrated that chemerin diminished neutrophil infiltration into the lung and generated proinflammatory cytokine while increasing recruitment of macrophages.[18] However, there were incompatible results in the association of asthma and systemic inflammation. Furthermore, if chemerin is an indicator of systemic inflammation, increased chemerin, and irisin values may be associated with allergic asthma. In this study, there was a significant difference in the chemerin levels between the control group and the asthma patients, but the chemerin level was even higher after omalizumab treatment (P = 0.124). This suggests that omalizumab treatment has no effect on serum chemerin levels. A recent study demonstrated that chemerin plays a protective role in allergic asthma by suppressing airway recruitment of inflammatory CD11c (+) CD11b (+) DCs through the inhibition of CCL2 secretion by active lung epithelial cells.[19] In the present study, we demonstrated

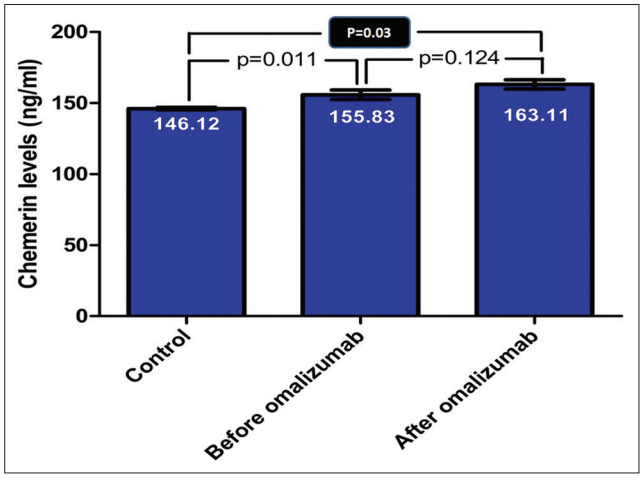

Figure 1: Chemerin levels (ng/mL) in healthy controls (Group-I) and patients (Group-IIA: before omalizumab and Group-IIB: after omalizumab) (mean ± standard error of the mean)

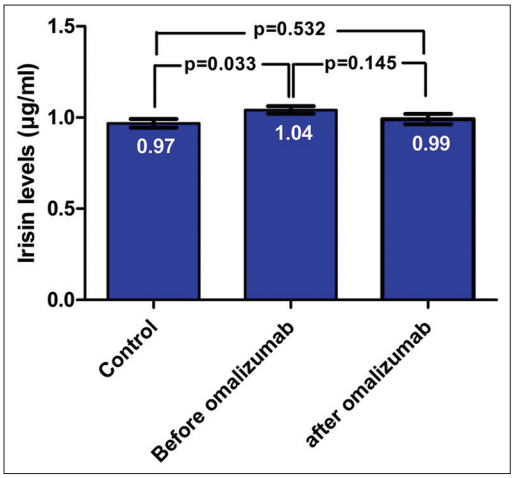

Figure 2: Irisin levels (pg/ml) in healthy controls and patients(Group-IIA and IIB) (mean ± standard error of the mean)

that NKC, DCs, and NKA levels were determined insignificant between before and after omalizumab periods. An unexpected higher level of chemerin after treatment is inconsistent with the previous report of Doyle *et al*.[20] who focused on the membrane-anchored chemerin receptor agonist and showed that chemerin decreased allergic airway inflammation, *in vivo*. This issue is under discussion.

Generally, the attachment of IgE in mast cell activation requires the cross-linking of FcεRI-bound IgE by an antigen or anti-IgE antibodies. In a transcriptome analysis of 8793 genes, the sensitization of mast cells with monoclonal IgE was found to be upregulated by 58 genes, more than twofold compared with their levels in unsensitized mast cells. These genes are composed of cytokines (IL-1 β, IL-6, and colony-stimulating factor 1), chemokines (CXCL8, CCL7, and CCL4), and chemokine receptors.[21-23] We found significant changes in IL-1 β, FE_{NO}, total IgE, ACT, FEV1, and FVC before the start of omalizumab treatment and 12 months after its onset, confirming that this is worth to consider as a therapeutic agent.

To sum up, we would like to conclude that, this is the first study to assess the level of circulating irisin and chemerin in asthma patients and their relationships with NKC, CSM, and NKA. We detected no significant changes neither in a variety of CSM depicting different cell subsets nor in multiple assays exploring cytokine expression and NKA. We think that the unchanged NKA level is a substantial indicator of drug safety against cancer development. We also believe that circulating chemerin and irisin may also have important roles in the relationship between severe allergic asthma and chronic inflammation. Further studies are needed to investigate whether the adipokine system has a role or chemerin and irisin are biomarkers in asthma.

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Conflicts of interest

There are no conflicts of interest.

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