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Regulatory T Cells (Tregs) Monitoring in Environmental Diseases

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A B S T R A C T

The prevalence of environmental diseases is increasing worldwide and these diseases are an onerous burden both to the individual and to the public health. Urban air pollution is a grave problem in majority of metropolises, which contain high levels of traffic congestion generating great amounts of genotoxic substances. The contribution of such environmental exposure to increase prevalence of many allergic, environmental diseases and multiple chemical sensitivity or other related syndromes, as a result of an abnormal immune response based on environmental damage of lymphocyte subsets, is marked. Benzene is one of the most important air pollutants that are emitted by oil industry, since they are involved in almost every refinery process. Volatile organic compounds (VOCs) are a major group of air pollutants and play a crucial role in ecological damages, disturbing the ecosystem and human health. The variability of pollutants is an important factor in determining human exposure to these chemicals. The immune system possess a capacity to distinguish between innocuous and harmful foreign antigens and controls this action by mechanisms of central and peripheral tolerance, where crucial role play regulatory T cells (Tregs). We analyzed the characteristics of human Tregs of inhabitants living near gasoline industry which have assessed moderate spyrometric tests and compared them with those situated in rural areas. Our data demonstrate that the chronic inhalation exposure increases the percentage of Tregs cells, but contrary those of inhabitants with decreased spirometry values have shown diminished number of Tregs, which may contribute to the new therapeutic approach of environmental diseases.

Key words: *chronic exposure to vapors, environmental diseases, innate immunity, regulatory T cells*

Introduction

The causative agents and their mechanisms that lead to the pathogenesis and developing of environmental diseases are still poor understood. The incidence of environmental diseases is increasing over the 30% in last two decades. These diseases include all disturbances caused by environmental, as well as behavioral factors. Environmental factors represent the group of changeable outdoor and indoor environments[1,2], while behavioral influences involve the augmentation in immunizations as a result of frequent infections, non regular use of antibiotics, diminished physical activities and decreased outdoor staying[3,4]. Inappropriate immune defense and consequently sensitization to possible allergens are the major reason to develop some type of environmental diseases[5]. The crucial role in peripheral T-cell tolerance have many factors as regulatory T cells (Tregs), anergy, activation-induced cell death, as well as genetic predisposition and environmental factors. Regulatory T cells (Tregs) play an important role in peripheral T-cell tolerance. Its deficiency leads to development of autoimmune syndrome: insulin dependent diabetes mellitus, tyreoiditis, colitis, arthritis, gastritis, as well as allergic disorders[6,7], while its activation suppresses activation of immune response against tumor and intracellular pathogens[8,9]. These immunoregulatory mechanisms of Treg cells are the subject of intensive investigation. The most specific and common marker that allow distinction regulatory from conventional T cells is forkhead box transcription factor (Foxp

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3)[10,11]. Predominant cell types that expressed Foxp 3 are surface markers double positive CD4+CD25+ and characteristic of subpopulations which are Foxp 3 negative are their absence of regulatory functions[12,13]. Furthermore, Tregs are able to suppress different cell types involved in immunity and inflammation by inhibition the proliferation, the blocking of NK and NKT-cell cytotoxicity, developing the immune surveillance pathways[14], changing the function and maturation of dendritic cells[15], as well as immunoglobulin production[16,17].

Subjects and Methods

In our examination we included 88 inhabitants devised in three groups: from industrial area, from rural fields and healthy volunteers. None of examiner was taking any systemically administered medications for at least 3 months before testing. All experiments have been approved by a local ethics committee.

Isolation of peripheral blood lymphatic cells (PBL)

Heparinized venous peripheral blood (10 mL) was layered on Ficoll/Hypaque density gradient and centrifuged for 20 min at 800×*g*. Cells were accumulated at the interface, washed twice in RPMI 1640 and resuspended at a final concentration of 1×10^6 peripheral blood lymphocytes per sample in fluorescent-activated cell sorting (FACS) buffer. Cell viability was checked by trypan blue.

Immunofluorescent staining and flow cytometry technique

Immunophenotypic profiles of peripheral blood T regulatory lymphocyte subsets were measured by using human regulatory T cell staining kit (eBioscience), which contains FITC CD4, APC CD25 and PE Foxp3, and prepared cells following the manufacture instructions. All samples had adequate isotypic controls. PBL were gated on the basis of forward and side scatter. A minimum of 10^4 cells was analyzed on a FACSCalibur (Becton Dickinson). Thresholds for positive staining were set at less than 2% using the negative control and percentages of positive cells were obtained by subtracting the value of the control.

Statistical analysis

The results were analyzed and showing using Microsoft Excel Statistical analyses were performed using by Mann-Whitney U-test. The differences were considered significant for $p<0.05$.

Results

People living in urban industrial fields have significantly decreased values of PIF (inspiratory peak flow), FIVC (inspiratory forced vital capacity), FIV1 (volume inspired in the first second of the test) and FVC (forced vital capacity) (Figure 1).

Furthermore, the inhabitants living in urban areas have more often obstructive/restrictive changes than those living in rural ones (Figure 2), indicating the influences of environmental factors on public health. 59.1% examine inhabitants from urban industrial areas have moderate and severe restriction/obstruction spirometry tests, compared to only 15% inhabitants from rural areas who have moderate restriction/obstruction spirometry findings.

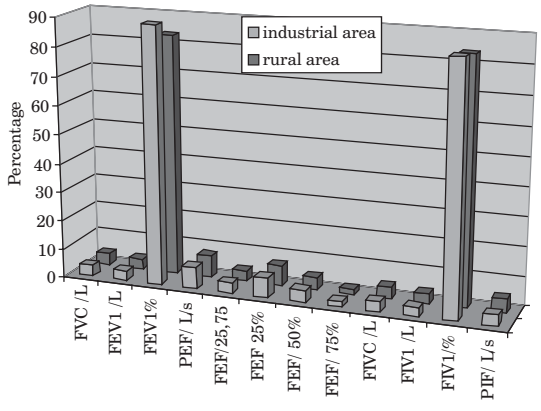

Fig. 1. Spirometry changes in inhabitants living in urban areas versus those living in rural fields.

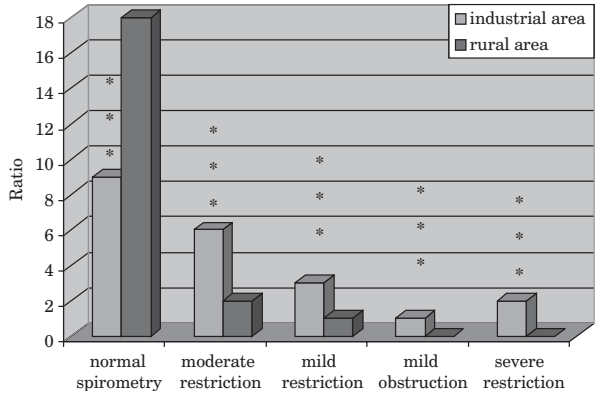

Fig. 2. Spyrometric changes ratio between industrial and rural areas.

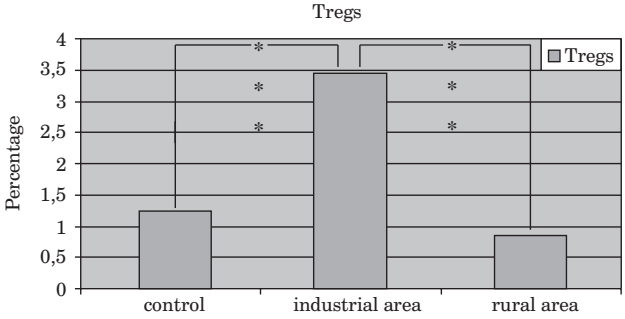

Fig. 3. The percentage of regulatory T cells (Tregs: CD4+CD25+ FoxP3+) in peripheral blood lymphatic cells (PBL) of inhabitants living in industrial area, compared to those from rural fields and with healthy volunteers, using flow cytometry technique.

A significant augmentation in a percentage of regulatory T lymphocytes was observed in peripheral blood of inhabitants living in industrial area, compared to those from rural fields (Figure 3).

Interestingly, we noticed significant changes in ratio Tregs/spirometry's tests between these examine groups (Figure 4). People from industrial areas have statistically significant increased values of Tregs than the control one, but in that group with spirometry's changes T regs have diminished values.

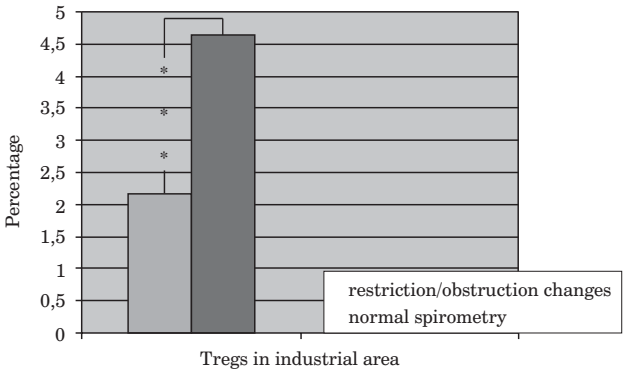

Fig. 4. Interactions between the spirometric changes and the percentage of regulatory T cells (Tregs) in inhabitants situated near industrial area.

Discussion and Conclusion

We wanted to determine the health impacts of these industrial pollutants which were assessed with spyrometric dynamic and static tests of inhabitants living in industrial areas, compared with those living in rural ones. Our results have shown significantly increasing values of Tregs in inhabitants situated near industrial areas, which were associated with the altered spyrometry's findings in inhabitants from urban areas, suggesting the great role of industrial emission on developing asthma and COPD and point to the possible predictive role of Tregs measuring in these subjects. We have shown for the first time the connections between restriction/obstruction changes and diminished values of Tregs in peripheral blood lymphocytes. Tregs have the ability to suppress allergic immune response induced by urban air pollution and their role in environmental diseases is marked. Treg cells monitoring in environmental diseases may play a crucial role in early diagnosis and prognosis of these diseases, as well as contributing to Treg cells-based immunotherapy.

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PRAĆENJE REGULACIJSKIH T STANICA (Tregs) U BOLESTIMA OKOLIŠA

S A Ž E T A K

Učestalost pojavljivanja bolesti okoliša raste širom svijeta i predstavlja veliko opterećenje kako za zdravlje pojedinca, tako i za javno zdravstvo u cjelini. Ispuštanje plinova industrijskih postrojenja i intenzivan promet u većini svjetskih metropola predstavlja velik problem koji doprinosi porastu učestalosti alergijskih i bolesti okoliša, mnogobrojnim raznovrsnim kemijskim preosjetljivostima, kao rezultat oslabljenog imunološkog odgovora uslijed oštećenja limfocitnih subpopulacija okolišnim čimbenicima. Benzen je jedan od najvažnijih zagađivala zraka, koji se otpušta u naftnoj industriji jer je uključen u gotovo svaki proizvodni proces. Hlapljivi organski spojevi predstavljaju glavnu grupu zagađivala zraka i imaju važnu ulogu u ekološkim oštećenjima, narušavajući ekosistem i ljudsko zdravlje. Velika različitost zagađivala predstavlja poteškoću u određivanju izloženosti ljudi tim kemijskim spojevima. Imunološki sustav posjeduje sposobnost razlikovanja vlastitog od tuđeg i upravlja ovom aktivnošću mehanizmima centralne i periferne tolerancije u kojima T regulacijske stanice (Tregs) imaju jednu od najvažnijih uloga. Ispitali smo karakteristike Tregs stanovnika koji žive u industrijskom području i uočili oslabljene spirometrijske funkcije, te ih usporedili s vrijednostima stanovništva ruralnih područja. Naši rezultati ukazuju da kronično izlaganje štetnim ispušnim plinovima povisuje vrijednosti Treg stanica. Međutim u osoba koje imaju snižene vrijednosti spirometrijskih mjerenja ove su vrijednosti također snižene, te možemo razmišljati o novom terapijskom pristupu bolestima okoliša.