



DETERMINATION OF IgG, IgA AND IgM IMMUNE COMPLEXES IN PLASMA OF ADOLESCENTS FROM CITIES OF STARA ZAGORA REGION WITH DIFFERENT LEVEL OF AIR POLLUTION

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ABSTRACT

Current study was designed to investigate immune parameters in blood plasma of adolescent persons chronically exposed to different degrees of ambient air pollutions. IgG, IgA and IgM circulating immune complexes (IC) were chosen as parameters of humoral immune response. The peripheral venous blood was taken from adolescents living in Stara Zagora, Kazanlak, Chirpan, Radnevo and Galabovo. The quantity of IgGIC, IgAIC and IgMIC in plasma samples was determined by CIF-ELISA. The significantly higher mean values of IgAIC were observed in students from Stara Zagora compared to students from other cities. Moreover, adolescents living in Stara Zagora showed significantly higher quantity of IgGIC then students from Radnevo and Galabovo. Adolescents living in Chirpan showed the highest quantity of IgMIC followed by students from Stara Zagora. The relatively highest concentrations of PM₁₀ and PM_{2.5}, SO₂ and NO₂ were measured in Stara Zagora. PM₁₀ concentrations exceeded the limit value in 50 % of all samples from Chirpan and in 40 % of samples from Stara Zagora. The level of PM_{2.5} was over the limit in 57.1 % of samples from Stara Zagora, followed by Chirpan (28.6 %). We concluded that air pollutants modulate humoral immune response activity in adolescents from the region investigated.

Key words: air pollution, IgM, IgG, IgA, immune complexes

INTRODUCTION

Excessive growth of human population and the accompanying pollution of water, air and soil are contributing to the increased number of human diseases worldwide. Currently an estimated 40% of world deaths are due to the environmental degradation (1).

Particulate matter (PM) present in ambient air is one of the six criteria pollutants, and the most important in terms of adverse effects on human health (2). Particulate matter is a mixture of solid and liquid particles. Solid

particles are composed of a core carbonaceous material and contain nitrates and sulfates, metals polycyclic aromatic hydrocarbons, alkanes and alcanoic acids, and biological components (endotoxin, pollen, fungal spores and etc.) (3).

Particulate matter is subdivided of coarse, fine and ultrafine particles. Coarse particles (PM₁₀) have a median aerodynamic diameter 10 µm or less. PM₁₀ fraction consists of insoluble minerals and biological aerosols. They are composed of aluminosilicate and other oxides of crustal elements and major sources including fugitive dust from roads, industry, agriculture and fly ash from fossil fuel combustion. Fine particles (median aerodynamic diameter 2.5 µm or less, PM_{2.5}) differ from PM₁₀ in origin and chemistry. They are composed of sulfate compounds, nitrate compounds, ammonium, hydrogen ion, organic compounds, metals (Pb, Cd, Ni, Cu,

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Mn and Fe) (2). PM can originate as a result of geographical conditions, weather, and seasonal patterns, or come from anthropogenic sources, including catalytic converters in cars, power plant emissions, and vehicle engine exhaust (3).

The air pollution also affects the big cities in Bulgaria, such as Sofia, Plovdiv, Varna, Stara Zagora, and others. During the last 20 years, atmospheric air over Stara Zagora region is among the most polluted in the country (4). Ambient air quality in Stara Zagora region is influenced by several groups of sources of emissions - Maritza East energy complex, located 40 km southwest of Stara Zagora, near to towns Galabovo and Radnevo; combustion plants to individual companies, hospitals, schools and others enterprises; the motor vehicles); combustion of different fuels in the houses, especially in winter and others. The main emissions from all these sources are SO₂, NO₂ and PM.

Many epidemiological studies demonstrated that the exposure of different air pollutants including PM has been related to risk of development of respiratory, cardiovascular and pulmonary diseases and cancer. Environmental pollutants could potentially lead to adverse health consequences through interaction with the immune system in several different ways. These include immune mediated functions of the humoral and cellular defense mechanisms in response to foreign substances. Immune alterations may give rise to immune enhancement, which result in autoimmune disease, hypersensitivity or allergy. On the other hand may lead to immune suppression, followed by increased incidence of infectious disease or neoplastic transformation (5)

Crosslinkage between soluble antigens and specific antibodies resulted in formation of a complex network called circulating immune complex. Immune complexes formation is a naturally occurring process in the body completing humoral immune response to an antigen. Depending of isotype of antibodies they are subdivided to IgM containing (IgMIC), IgG containing (IgGIC) and IgA containing (IgAIC). Immune complexes may also contain complement subfragments primarily C3b and C4b. Generally, immune complexes are transported by erythrocytes via CR1 to the liver, where are effectively removed by mononuclear phagocytes through complement receptors (CR3 and CR4) and receptors for Fc fragment of IgG antibodies

(FcγR). This cycle of IC formation and clearance is an important part of acquired immunity, ensuring removal or processing of antigens. However, there is growing body of evidence that the immune complexes may function as a trigger of many autoimmune diseases and immune complexes mediated inflammation. In particular elevated levels of IgMIC and IgAIC are involved in development of diseases as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), systemic sclerosis (PSS), IgA nephropathy, vasculitis and other (6-8).

Adolescents are a sensitive group for adverse effects of atmospheric pollutants on the immune system. In this study we investigated the changes of serum level of circulating immune complexes containing IgM, IgG and IgA antibodies (IgGIC, IgMIC, IgAIC), as biomarkers of the humoral immune response, in blood plasma of adolescent persons chronically exposed to different degrees of ambient air pollutions in Stara Zagora region.

MATERIALS AND METHODS

Subjects

For the study, cities of Stara Zagora region with different air pollution were chosen. Selected cities were Stara Zagora - highly industrialized city with intense traffic, Kazanlak - with relatively underdeveloped industry, Chirpan - little rural town, Radnevo and Galabovo located close to the energy complex Maritza East.

We investigated groups of 18-year old healthy adolescents, grew up in those cities. The selection of adolescents of the same age was in order to create relation between long time exposure to the local environmental pollutions and time-dependent accumulation of harmful health effects leading to aberrant immune response. 40 volunteers living in Stara Zagora, 27 from Kazanlak, 27 living in Chirpan, 18 from Radnevo and 24 living in Galabovo were recruited for this study. The informing consent was obtained from each participant. The peripheral venous blood (3 ml) was taken by venepuncture and collected in sterile tubes with ethylenediamine tetraacetic acid (EDTA). The plasma samples were separated at room temperature within 4 h of collection and stored at -70°C until use.

Determination of IgMIC, IgGIC and IgAIC

The assessment of circulating IgG, IgM and IgA immune complexes in human plasma was

performed by homemade CIF-ELISA according to Stanilova and Slavov, 2001 (9) and Stanilova and Slavov, 2003 (10). Nunc's maxisorp immunomodules was used for the performance of CIF-ELISA. CIF were isolated in our laboratory from total water extract of the seeds of parasitic plant *Cuscuta europea*, as described previously by Zhelev et al., 1994 (11). For the detection of human IgM, IgG and IgA, goat anti-human IgM, goat anti-human IgG and goat anti-human IgA respectively, conjugated with peroxidase (Sigma) were used. The color reaction developed was measured at OD 492 by an ELISA reader.

Determination of PM₁₀, PM_{2.5}, SO₂ and NO₂.

Monitoring points: For the purposes of the study, 5 points were determined as follow: Point Code (PC)101 (Stara Zagora, urban background station); PC 102 (Sulitsa village, regional station); PC 103 (Chirpan, urban background station); PC 104 (Kazanlak, urban background station) and PC 105 (Gurkovo, urban background station) – control point.

Air samples were collected by hand during the period from March to December 2010, once a month for a point. For determination of particulate matter (PM), TECORA "Echo PM" device was used. For determination of SO₂ and NO₂, "Bravo M-Basic" device was applied. The equipment was calibrated (manually and automatically) before each sampling.

Air samples were taken as follows:

- (a) for contents of NO₂ and SO₂: on two consecutive days at daylight, in four time ranges (8:00 to 9:00 h, 10:30 to 11:30 h, 13:30 to 14:30 h and 15:30 to 16:30 h) as in the National Air Monitoring System; and
- (b) for content of PM₁₀ and PM_{2.5}: once, by continuous sampling over 24 hours.

Nitrogen dioxide (NO₂) was determined directly by a spectrophotometric method with alfa-naftilamin (Bulgarian State Standard, BSS 17.2.4.22-83). Light absorption was measured at $\lambda = 540$ nm and thickness of 10 mm. Total number of samples taken and analyzed was 368.

Sulphur dioxide (SO₂) was determined directly by a spectrophotometric method (BSS 17.2.4.17-83). The method is based on the absorption of sulfur dioxide from the air in a solution of sodium or potassium tetrahydromercurat. Light absorption was

measured at $\lambda = 580$ nm and thickness of 10 mm. The total number of samples taken and analyzed was 367.

PM₁₀ and PM_{2.5} fractions were determined directly by gravimetric method (BSS 17.2.4.20; BS EN 12341). The total number of samples taken and analyzed was 50 for PM₁₀ and 35 for PM_{2.5}.

Statistical analysis

The data are expressed in OD 492 units as means and standard error of the mean. Student's t-test was used to determine the statistical differences between mean values. Differences were considered significant when the P value was less than 0.05.

RESULTS

Serum level of IgMIC, IgGIC and IgAIC

Changes in plasma level of IgMIC in students from different cities are presented in **Fig. 1**. Adolescents living in Stara Zagora and Chirpan showed an increased level of IgMIC compared with their counterparts living in Radnevo, Galabovo and Kazanlak. However, this difference reached significant degree only about students living in Chirpan ($p=0.004$ for Chirpan vs. Kazanlak; $p=0.03$ - Chirpan vs. Galabovo; $p=0.01$ - Chirpan vs. Radnevo). There were no significant differences between adolescents living in Radnevo, Galabovo and Kazanlak.

Highest level of IgGIC was observed in plasma taken from adolescents living in Stara Zagora (**Fig. 2**). IgG containing immune complexes in students from Stara Zagora were significantly higher in comparison with IgGIC found in persons from Galabovo ($p=0.009$) and Radnevo ($p=0.02$). There was no statistical significance between adolescents from Stara Zagora, Kazanlak and Chirpan. Adolescents from Galabovo showed significantly lower IgGIC than their counterparts from Kazanlak.

Fig. 3 shows an overview of evaluated IgA immune complexes in students from selected cities. Results clearly demonstrated that students from Stara Zagora have the highest level of IgA containing immune complexes. Statistically significant increase of IgAIC was observed for group from Stara Zagora in comparison with youth living in areas of Kazanlak ($p=0.000001$), Chirpan ($p=0.000001$), Galabovo ($p=0.0026$) and Radnevo (0.000000). Adolescents from Radnevo have significantly lowest level IgAIC in comparison with their counterparts from other cities.

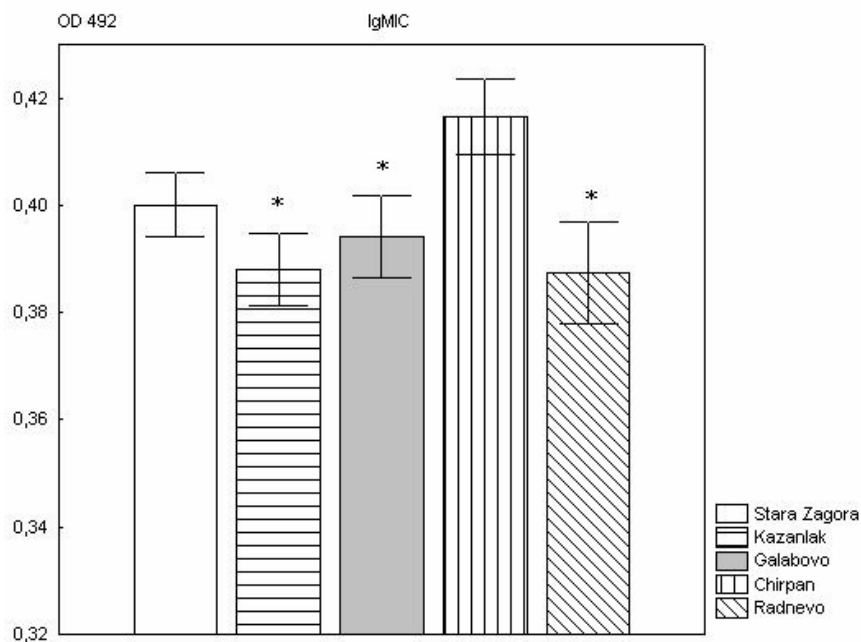


Fig. 1. Changes in plasma level of IgMIC in students from Stara Zagora, Kazanlak, Galabovo, Chirpan and Radnevo. * $P < 0.05$ - Chirpan vs. Kazanlak, Galabovo and Radnevo.

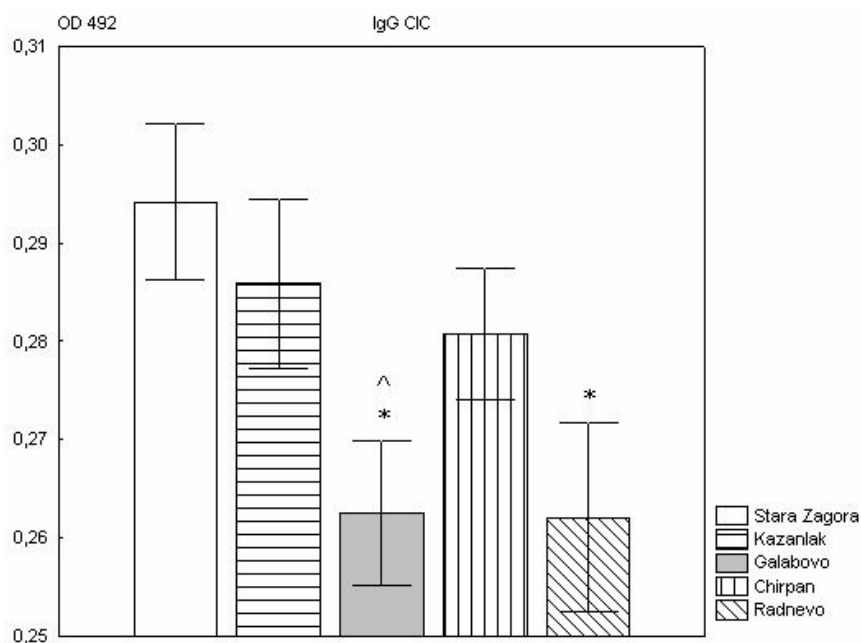


Fig. 2. Changes in plasma level of IgGIC in students from Stara Zagora, Kazanlak, Galabovo, Chirpan and Radnevo. * $P < 0.05$ - Stara Zagora vs. Galabovo and Radnevo; ^ $P < 0.05$ - Kazanlak vs. Galabovo.

Established contents of SO_2 , NO_2 , PM_{10} and $\text{PM}_{2.5}$ in air

The data expressed as average hourly concentrations for SO_2 and NO_2 , and as average 24 hourly concentrations for PM_{10} and $\text{PM}_{2.5}$ are presented in Fig. 4. All detected concentrations of SO_2 were lower from 6.3 to 1207 times than the hourly allowed rate (350

$\mu\text{g}/\text{m}^3$) for the protection of human health (Regulation No 12/2010). Similar tendency was established for the content of NO_2 . The levels of NO_2 were lower 6.2 to 526 times than the hourly rate for the protection of human health, which is equal to 200 $\mu\text{g}/\text{m}^3$ (Regulation No 12/2010). The only differences in average values for points Sulitsa and

Kazanlak are statistically proven at $P < 0.05$. Average, minimum and maximum concentrations of SO_2 and NO_2 varied in a narrow range for the different points. However, the highest was the content of SO_2 in point 102-Sulitsa, followed by points 105-Gurkovo,

101-Stara Zagora and 104-Kazanlak. The arrangement of points concerning specified levels of NO_2 is in another sequence - the highest content was at point 104-Kazanlak, followed by points 105-Gurkovo, 101-Stara Zagora, 103-Chirpan and 102-Sulitsa.

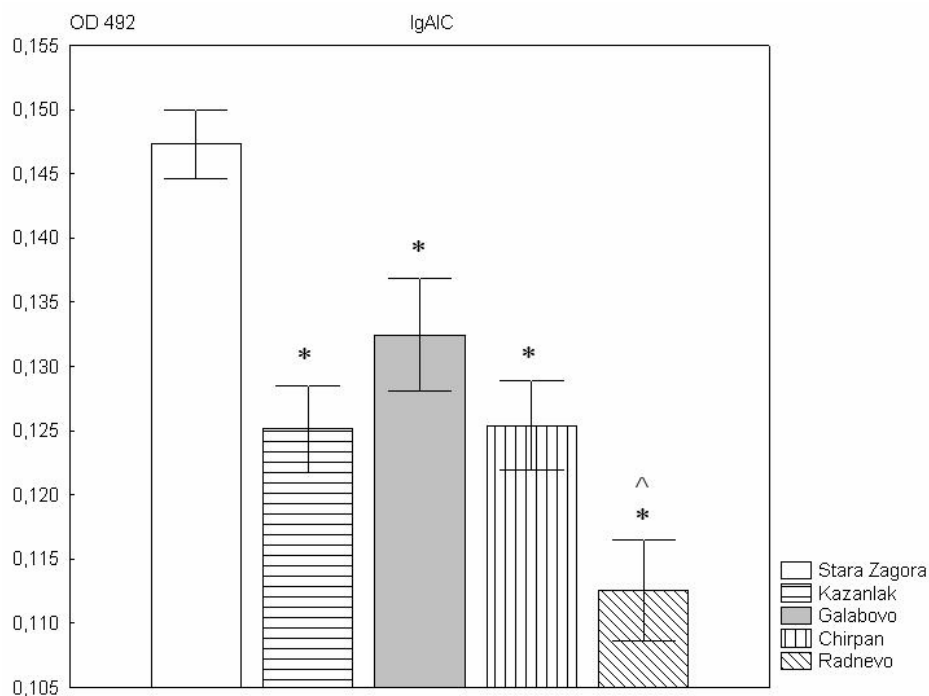


Fig. 3. Changes in plasma level of IgA1C in students from Stara Zagora, Kazanlak, Galabovo, Chirpan and Radnevo. * $P < 0.05$ – Stara Zagora vs. Kazanlak, Galabovo, Chirpan and Radnevo. ^ $P < 0.05$ – Galabovo vs. Radnevo.

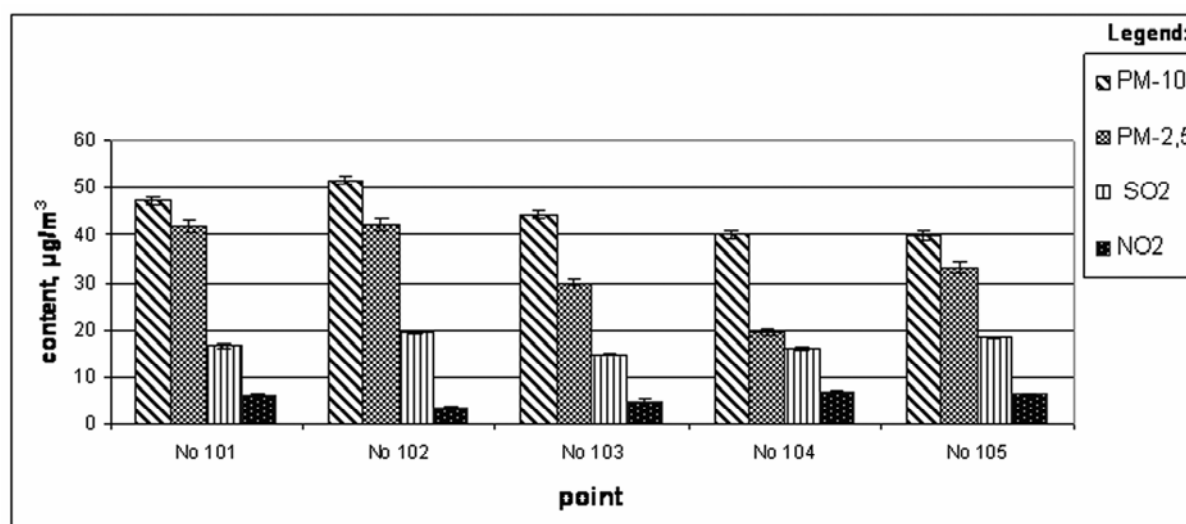


Fig. 4. Average hourly concentrations of SO_2 and NO_2 and average daily concentrations of PM_{10} and $\text{PM}_{2.5}$ in ambient air at 5 points: 101 - Stara Zagora, urban background station; 102 - Sulitsa village, regional station; 103 - Chirpan, urban background station; 104 - Kazanlak, urban background station and 105 - Gurkovo, urban background station, control point. * MAC: PM_{10} – $50 \mu\text{g}/\text{m}^3$; $\text{PM}_{2.5}$ – $30 \mu\text{g}/\text{m}^3$; SO_2 – $350 \mu\text{g}/\text{m}^3$; NO_2 – $200 \mu\text{g}/\text{m}^3$

The content of PM₁₀ was in the range from 7.27 µg/m³ at point 104-Kazanlak to 99.3 µg/m³ in point 102-Sulitsa. Average concentrations for the considered period exceeded the limit one of 50 µg/m³ (Regulation No 12/2010) only in point 102-Sulitsa. At the other points, average values were under the limit.

Daily average concentrations of PM_{2.5} were in the range from 4.32 µg/m³ at point 103-Chirpan to 98.3 µg/m³ in point 101-Stara Zagora. The highest level at the limit concentration 30 µg/m³ (Regulation No 12/2010) was found in point 101-Stara Zagora. In relative number of days with high levels PM_{2.5}, the most polluted air was in points 102 (66.7 % of the days), followed by points 101-Stara Zagora.

DISCUSSION

Analysis of the data of air quality gives reason to assert that PM₁₀ and PM_{2.5} have been the main atmospheric pollutants around the five monitoring points. The complex air quality assessment based on these two indices defined the most polluted air in point 101-Stara Zagora. We observed the most pure air at points 104-Kazanlak.

Current study was designed to investigate immune parameters in blood plasma of adolescent persons chronically exposed to different degrees of ambient air pollutions. IgG, IgA and IgM circulating immune complexes (IC) were chosen as parameters of humoral immune response.

Very few studies about the harmful effects of airborne pollutants on the developing immune system of children and adolescents are conducted in Bulgaria. In one study, Leonardi et al., 2000 (12) investigated the changes of some immune biomarkers in children exposed to atmospheric pollutants. They survey includes 366 children living in 17 towns of Bulgaria, the Czech Republic, Hungary, Poland, Romania and Slovakiya. The authors showed that the number of B, CD4+, CD8+, and NK lymphocytes and total IgG increased with increasing concentration of PM. Our results were in same direction. We demonstrated that students from Stara Zagora have the highest quantity of IgGIC in comparison with their counterparts from other cities. We want to emphasize that most air pollution was established in Stara Zagora,

followed by the adjacent area around Sulitsa. Especially, PM_{2.5} concentrations exceeded the limit value in 51.7% of the tested days for Stara Zagora and 66.7% for Sulitsa. Several reports have linked exposure to urban pollutants (particulate matter and SO₂) with elevated levels of IgG antibodies. For example, the study of Tomei et al., 2004 (13) demonstrated increased serum level of IgG antibodies against HSV-1 in traffic policemen compared with controls. Effect of SO₂ were demonstrated by Syed et al., 2000 (14) in their study. The authors showed that high atmospheric pollution with SO₂ is related with increased IgG value in sera of industrial workers. Our result extended these data and demonstrated that at least part of enhanced IgG is included in immune complex.

Many epidemiological studies demonstrated that ambient air pollution particles can adversely affect human health and exposure to different air pollutants form, like solid particles, liquid droplets, or gases has been related to risk of cardiovascular and pulmonary diseases and cancer (1). The effects of airborne pollutants on the immune system have been most widely studied in the respiratory tract (15, 16). The respiratory tract is the largest body surface exposed to the external environment, and the inflammatory response in the lungs is highly prevalent (17). In the lower airways inhaled particles are phagocytized by bronchoalveolar macrophages. Previous studies have shown that PM₁₀ and PM_{2.5} have various cellular effects on alveolar macrophages, including induction of cytokine production (18). Cytokines generated during inflammatory processes (IL-6, IL-1, TNF-α) in the respiratory tract may influence systemic response of the immune system, proliferation of activated antigen specific B cells and exacerbated antibody production.

IgA play a significant role in mucosal immunity, including respiratory immune response. In this study IgA containing immune complexes (IgAIC), as a marker for mucosal immunity, were determined in plasma of selected groups of students by ELISA. Our results demonstrated significantly higher level of IgAIC in group of Stara Zagora than in groups of less polluted with PM cities: Kazanlak, Galabovo, Chirpan and Radnevo. Prevalence of IgA containing IC in blood combined with secretory and mucosal activities of IgA suppose involvement of these

complexes in airway hyperresponsiveness. Similar results are demonstrated also by other investigators. Elevated levels of IgA antibodies are detected by Hadnagy et al., 1996 (5) in persons living in Dortmund (highly industrialized region) compared with persons living in reference area Borken. Recently, enhanced IgA synthesis and changes of IgE/IgA and IgE/IgG4 ratio are connected with changes of allergen-specific Th2 to Treg cell predominance and decreased allergic response (19). Therefore, these data confirm our suggestion that enhanced IgAIC in adolescents living in Stara Zagora are involved in developing allergic response.

On the other hand, enhanced antibody synthesis and delayed immune complexes clearance may function as a trigger of many autoimmune diseases and immune complexes mediated inflammation. Pathogenic properties of immune complexes are depending predominantly of their size, isotype of antibodies, complement activation and function of CR1. For example, apoptotic cells are preferentially recognized by IgM antibodies which form large immune complexes and activate the complement system. As a consequence they are removed by interaction with erythrocyte CR1 followed by interaction with CR1, CR3 and CR4 of phagocytic cells in liver and spleen. Significantly higher level of IgMIC in patients with rheumatoid arthritis and progressive systemic sclerosis are demonstrated in study of Stanilova and Slavov, 2003 (10). IgG antibodies usually elaborated in response to foreign antigens and frequently form complexes of smaller sizes. They also activate the complement system and removed by CR1 in collaboration with FcγR of phagocytic cells. IgA immune complexes cannot cause complement activation via classical pathway, but they can initiate complement activation via alternative pathway. Effectiveness of their binding to erythrocytes is significantly lower than corresponding activity of IgG immune complexes of the same size. Liver and spleen macrophage uptake of IgAIC is less effective than that of IgGIC, and IgAIC were found in lungs and kidneys (glomeruli) of the experimental animals (20). Deposition of IgAIC on the vascular wall and its presence in kidney glomeruli is typical for acute vascular purpura (Henoch-Schönlein syndrome) and IgA nephropathy (21, 22). As mentioned above, in our previously published

investigation, we found that in RA and PSS groups mainly persists IgMCIC, while in SLE prevails IgG and IgACIC (10).

In conclusion, we demonstrated that air pollutants modulate humoral immune response by altering class switching of antibody production. Increased levels of IgA immune complexes in students from Stara Zagora can serve as a biomarker for exacerbation of humoral immune response and are a prerequisite for the development of allergic and autoimmune diseases.

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