

# Determination of Enzyme Matrix Metalloproteinases-9 and Immune Status as Indicators of Development of the Environmental Diseases

Aleksandar Bulog<sup>1</sup>, Vladimir Mićović<sup>1</sup>, Petra Šuljić<sup>1</sup> and Ines Mrakovčić-Šutić<sup>2</sup>

<sup>1</sup> Department of Public Health, School of Medicine, University of Rijeka, Rijeka, Croatia

<sup>2</sup> Department of Physiology and Immunology, School of Medicine, University of Rijeka, Rijeka, Croatia

## ABSTRACT

*The majority of environmental diseases are multifactorial airway illnesses, including genetic background and exposure to different kind of airborne irritants and allergens. Altered lifestyle and changes in environmental exposures contribute to the occurring of these diseases. The term of environmental illnesses includes the disease primarily caused by pollution of air and water, chemical and physical agents, radiation, contaminated food and direct contact with the toxins we are exposed to natural and/or working environment. The members of the matrix metalloproteinase (MMP) family are involved in the pathogenesis of COPD. MMPs comprise a large family of structurally related zinc metalloendopeptidases with different substrate specificities and possibilities to degrade protein constituents of the extracellular matrix. We investigated immunological status and level of MMP-9 in workers occupationally exposed to volatile aromatic hydrocarbons compared to urban residents and rural areas. The phenotypic profiles of peripheral blood lymphocytes were done by flow cytometry. The method of enzyme immunoassay (ELISA) was used to determine enzyme expression of matrix metalloproteinase-9 (MMP-9). The occupationally exposed group had a significantly elevated level of enzyme MMP-9 in the urine, accompanied with augmentation of cells of innate immunity in peripheral blood, which could contribute to the monitoring, early detection of environmental diseases and consequent earlier and more effective treatment.*

**Key words:** environmental disease, enzyme metalloproteinase-9 (MMP-9), innate immunity, NKT cells.

## Introduction

Nowadays, the focus of interest of many research groups is the change in environmental factors, which play a crucial role in the development of the environmental diseases<sup>1</sup>. Many enzymes with protective and regenerative role change their features, as a direct consequence of the increasing pollution. Matrix metalloproteinase-9 (MMP-9) or gelatinase B belongs to the family of enzymes that commonly called matrix metalloproteinases. Today, there are a total of 24 more or less explored matrix metalloproteinases, which are primarily divided by the structure their domain. Gelatinase B is synthesized in many cell types, such as keratinocytes, monocytes, tissue macrophages, polymorphonuclear leukocytes and many types of tumor cells, and the intensity of release of active enzyme is dependent on the amount of the enzymes

stored in granules of these cells<sup>2–5</sup>. MMPs comprise a large family of structurally related zinc metalloendopeptidases with different substrate specificities and possibilities to degrade protein constituents of the extracellular matrix. Increased expression of the enzyme matrix metalloproteinase-9 (MMP-9) in the human body is associated with protective and regenerative effects on the lung epithelial cells in serious damage of the lung epithelium caused by chronic obstructive pulmonary disease (COPD)<sup>6</sup>. Statistically significant expression of matrix metalloproteinase-9 is demonstrated in various cases of lung cancer, inflammatory conditions, where it is as a proteinase involved in many processes of proliferation, differentiation and migration of mast cells and its release is regulated by secreted cytokines such as tumor necrosis

factor (TNF) and interferon (IFN)<sup>7</sup>. Expression of matrix metalloproteinase-9 (MMP-9) has been recognized in direct contact with T lymphocytes, monocytes<sup>8</sup> and mast cells<sup>9</sup> and during the increased migration of neutrophils and eosinophils in severe damage of lung tissue<sup>10,11</sup>. Qualitatively and quantitatively determination of the expression of the enzyme metalloproteinase-9 (MMP-9) is used for estimation of lung disease, and as a method of determination the exact biological indicators (biomarkers) of increased risk of exposure to carcinogens and toxic environmental contaminants and possible development of the environment diseases in the form of severe inflammatory conditions (COPD) and/or various forms of lung cancer<sup>12,13</sup>. Regulation of secretion of matrix metalloproteinase-9 is best examined in the case of neutrophils, as representatives of cells of non-specific immunity with phagocytic characteristics as well as with ability to directly kill target cells via different mechanisms mediated by lizozim or defenzin. As inflammatory cells, neutrophils are the first line of immunological defense of the human organism to various environmental factors, and in the inflammatory process, matrix metalloproteinase-9 plays a key role. Like other highly specialized immune cells, T and B lymphocytes, monocytes / macrophages, dendritic cells and NK cells, synthesize and reconstructed matrix metalloproteinase-9 in their granules. Synthesis and partitioning of metalloproteinase-9 results in a rapid immune defense in the form of releasing large amounts of active enzyme in a time of just 15 minutes without the need for processes such as transcription and translation<sup>14–16</sup>. According to the newly scientific research which are related to the detection of toxic effects of environmental pollutants on the human immune system<sup>17</sup> there are the possibility for better prevention of public health.

## Subjects and Methods

We investigated immunological status and level of MMP-9 in workers occupationally exposed to volatile aromatic hydrocarbons (shipyard Viktor Lenac in Rijeka and Kraljevica in Kraljevica) compared to urban residents (Rijeka) and rural areas (Mali Lošinj). As the samples in this paper we used first morning urine and peripheral venous blood of subjects who, according to a number of criteria (non-smoker, work-able adults without acute and chronic illnesses) as members of test groups have all goals and criteria for research. In our research, we included residents of the City of Rijeka who live and work in a typical metropolitan area (urban area), residents who live and work on the island Mali Lošinj as characteristic of unpolluted areas (rural areas), and occupationally exposed subjects (occupationally exposed subjects), which the daily coating of marine construction (shipbuilding) are exposed to the investigated compounds via inhalation and contact, using paint and varnish that has been proven (Article 129 paragraph 3 of the Environmental Protection Act, »Narodne novine«. 110/07-Registar environmental pollution) in its composition

have compounds in the form of organic solvent. The phenotypic profiles of peripheral blood lymphocytes were done by flow cytometry. The method of enzyme immunoassay (ELISA) was used to determine enzyme expression of matrix metalloproteinase-9 (MMP-9). Statistical data were calculated using computer program Statistica 7.1. The experiments were provided the respect of fundamental ethical and bioethical principles such as personal integrity (autonomy), justice, benevolence and safety, all in accordance with the Nurnberg Code and the latest revision of the Helsinki Declaration<sup>18</sup>.

## Results

In Figure 1 we can notice that the occupationally exposed group had statistically significantly elevated levels of MMP-9 in the urine.

The concentrations of the enzyme MMP-9 were significantly elevated in the population of the city of Rijeka in relation to Mali Lošinj ( $p < 0.05$ ) in relation to workers and the shipyard Viktor Lenac Kraljevica statistical significance was at  $p < 0.01$ .

Inhabitants of City of Rijeka and Mali Lošinj, as well as workers in the shipyard Viktor Lenac and Kraljevica have the normal values of total T lymphocytes (CD3+), helper T cells (CD4+) and cytotoxic/suppressive T cells (CD8+) (Figure 2–5).

Figure 6 shows a decrease in the percentage of NK cells in the workers and the shipyard Viktor Lenac But the prince mentioned the reductions are small and sporadic, and not statistically significant.

However, the proportion of NKT cells (CD3+ CD16+ 56+) was significantly higher in shipyard workers and residents of urban areas ( $p < 0.05$ ) compared with the control group and residents of rural areas (Mali Lošinj) (Figure 7).

## Discussion and Conclusion

The development of modern cities is possible only with great sources of energy, because the rapid development of various forms of industry use petroleum as the main energy source. In the city of Rijeka, port, road and rail traffic has developed in past few decades accompanied with very strong oil and gas industry where is the

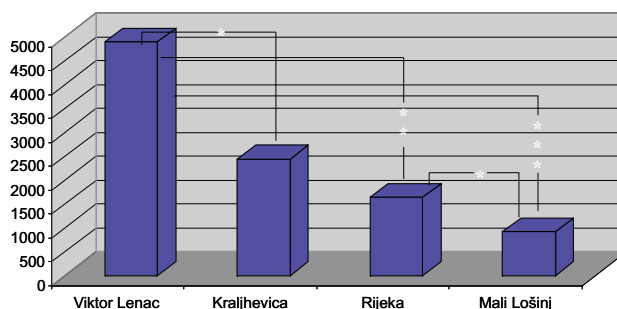


Fig. 1. Average concentrations of the enzyme MMP-9 in urine (pg/mL).

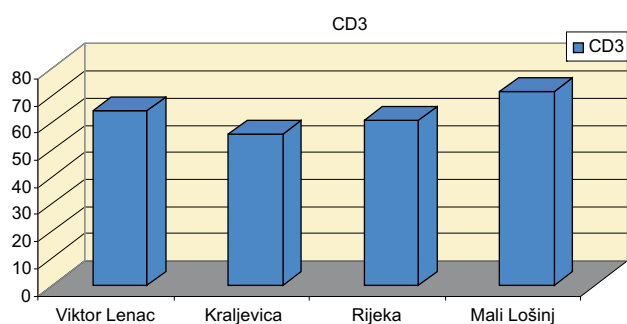


Fig. 2. Changes in the concentration of CD3+ cells in workers of shipyard Viktor Lenac and Kraljevica and the inhabitants of Rijeka and Mali Lošinj.

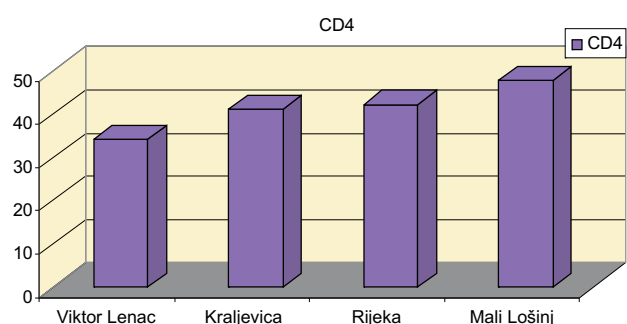


Fig. 3. Changes in the concentration of CD4+ cells in workers of shipyard Viktor Lenac and Kraljevica and the inhabitants of Rijeka and Mali Lošinj.

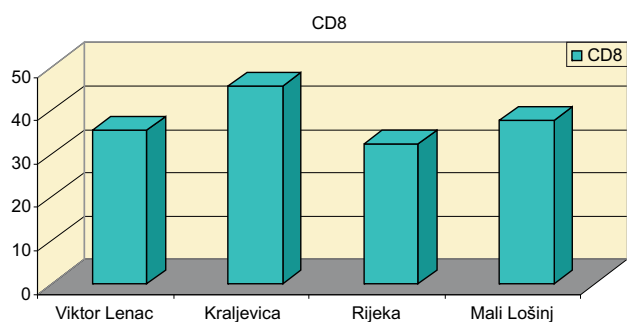


Fig. 4. Changes in the concentration of CD8+ cells in workers of shipyard Viktor Lenac and Kraljevica and the inhabitants of Rijeka and Mali Lošinj.

main feature of the release of potentially hazardous compounds in various ways into the process of environmental balance and affect human health. Many exhaust compounds formed by the work of the industry that influence the processes in the human body leading to disease pathogenesis and development of the environment, are still insufficiently known. The frequency of development of environmental disease in developed countries is increasing, and they represent a burden for the individual and public health in general. These are also the main reason why this disease should be identified as early as possible, and the factors that underpin their development should try to eliminate or reduce their adverse impact as

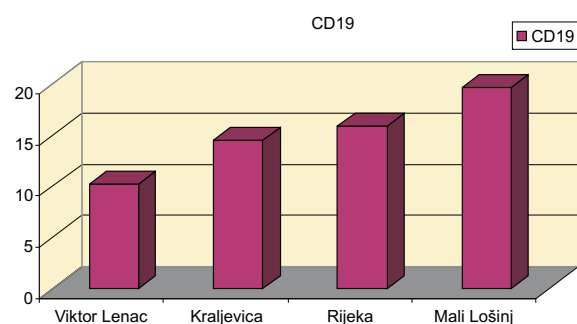


Fig. 5. Changes in the concentration of CD19+ cells in workers of shipyard Viktor Lenac and Kraljevica and the inhabitants of Rijeka and Mali Lošinj.

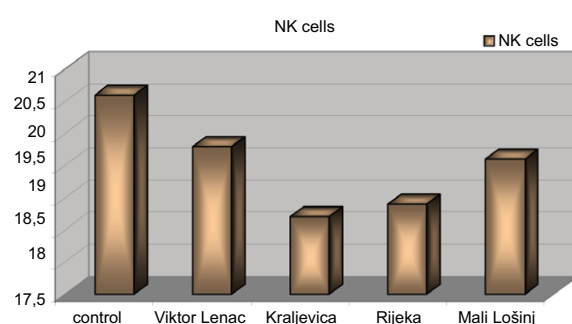


Fig. 6. Changes in the concentration of NK cells (CD3-CD56+) in workers in the shipyard Viktor Lenac and Kraljevica and population of Rijeka and Mali Lošinj.

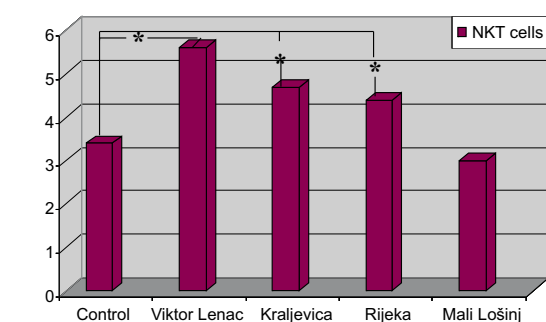


Fig. 7. Changes in the concentration of NKT cells (CD3+ CD56) in workers in the shipyard Viktor Lenac and Kraljevica and population of Rijeka and Mali Lošinj.

far as the highest possible<sup>19,20</sup>. Expression of the enzyme matrix metalloproteinase -9 (MMP-9) was significantly elevated in both the professional disadvantaged group, as well as in subjects of urban areas. The percentage of NK and NKT cells is reduced in peripheral blood of subjects occupationally exposed to volatile aromatic hydrocarbons.

## Acknowledgements

This work was supported by grants from the Croatian Ministry of Science (No 0621341-0308 and 0620096-0094).

## REFERENCES

1. MILLER FW, Adv Exp Med Biol, 711 (2011) 61. — 2. EGEBLAD M, Nature Rev Cancer, 2 (2002) 161. — 3. ZHU HJ, BURGESS A W, Mol Cell Biol Res Commun, 4 (2001) 321. — 4. MA Z, QIN H, BENVENISTE EN, J Immunol, 167 (2001) 5150. — 5. KITAHARA M, ICHIKAWA M, KINO-SHITA T, SHIOZAWA S, SHIGEMATSU H, KOMIYAMA A, Kidney Blood Press Res, 24 (2001) 18. — 6. PUCHELLE E, ZAHM JM, TOURNIER JM, CORAUX C, Proc Am Thorac Soc, 3 (2006) 726. — 7. GIROLAMO ND, INDOH I, JACKSON N, WAKEFIELD D, MCNEIL HP, YAN W, GECZY C, JONATHAN P, TEDLA A, TEDLA N, Immunology, 177 (2006) 2638. — 8. LACRAZ S, ISLER P, VEY E, WELGUS HG, DAYER JM, J.Biol.Chem, 269 (1994) 22027. — 9. BARAM D, VADAY GG, SALAMON P, DRUCKER I, HERSHKOVIZ R, MEKORI YA, J Immunol, 167 (2001) 4008. — 10. OKADA S, KITA H, GEORGE TJ, GLEICH GJ, LEIFERMAN KM, Am. J. Respir Cell Mol Biol, 17 (1997) 519. — 11. KECK T, BALCOM JH, FERNANDEZ DEL CASTELLO C, ANTONIU BA, WARSHAW AL, Gastroenterology, 122 (2002) 188. — 12. KOC M, EDIGER D, BUDAK F, KARADAG M, ORAL HB, UZASLAN E, EGE E, GÖZÜ RO, Journal of Experimental and Clinical Oncology, 2 (2006) 92. — 13. LUND AK, KNUCKLES TL, AKATA CO, SHOHET R, MCDONALD J, GIGLIOTTI A, SEAGRAVE JC, CAMPEN MJ, Toxicological Sciences, 95 (2007) 485. — 14. OPDENAKKER G, VAN DEN STEEN PE, VAN DAMME J, Trends Immunol, 22 (2001) 571. — 15. BARTHOLOMÉ EJ, WILLEMS F, CRUSIAUX A, THIELEMANS K, SCHANDENE L, GOLDMAN M, J Interferon Cytokine Res, 19 (1999) 471. — 16. MASURE S, PROOST P, VAN DAMME J, OPDENAKKER G, Eur J Biochem, 198 (1991) 391. — 17. MIČOVIĆ V, VOJNIKOVIĆ B, BULOG A, COKLO M, MALATESTINIĆ D, MRAKOVČIĆ-SUTIĆ I, Coll Antropol, 33 (2009) 743. — 18. WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI. ETHICAL PRINCIPLES FOR Medical Research Involving Human Subjects. Available from: URL: <http://www.wma.net/e/>; assessed: 12.02. 2009. — 19. FRIESEN MC, DAVIES HW, TESCHKE K, OSTRY AS, HERTZMAN C, DEMERS PA, Epidemiology, 18 (2007) 88. — 20. BIGGERI A, LAGAZIO C, CATELAN D, PIRASTU R, CASSON F, TERRACINI B, Epidemiol Prev, 30 (2006) 5.

### I. Mrakovčić-Šutić

Department of Physiology and Immunology, University of Rijeka, School of Medicine, Braće Brancheeta 20, 51000 Rijeka, Croatia

e-mail: [inesm@medri.hrr](mailto:inesm@medri.hrr)

## ODREĐIVANJE ENZIMA MATRIX METALLOPROTEINAZE-9 I IMUNOLOŠKOG STATUSA KAO POKAZATELJA RAZVOJA BOLESTI OKOLIŠA

### SAŽETAK

Većina bolesti okoliša predstavlja bolesti različitih uzroka, uključujući i genetske uzročnika, kao i izloženost različitim vrstama iritansa i alergena. Promjena načina života i promjena u okolišu doprinijeli su pojavi boleasri. Pojam zaštite od pojavljivanja bolesti okoliša, uključuje prvenstveno zaštitu od bolest uzrokovanih zagađenjem zraka i vode, kemijskih i fizikalnih agenasa, zračenja, zagađene hrane i izravan kontakt s toksina fizičkim i/ili radnim okruženjem. Članovi matrix metaloproteinaze (MMP) obitelji su uključeni u patogenezu kronične opstruktivne bolesti pluća (KOBP). MMPs obuhvaćaju veliku obitelj strukturno povezanih enzima koji oslobađaju cink metaloendopeptidasu s različitim podlogama specifičnosti i s mogućnosti razgradnje bjelančevinastih sastojaka izvanstaničnog matriksa. Istraživali smo imunološki status i razinu MMP-9 u radnika profesionalno izloženih hlapljivim aromatskim ugljikovodicima u odnosu na gradsko stanovništvo i ruralna područja. Fenotipske osobitosti limfocita periferne krvi smo ispitali protočnom citometrijom. Imunohistokemija (ELISA) je korištena za određivanje enzima matrix metaloproteinaze-9 (MMP-9). Profesionalno izložena grupa je imala značajno povišenu razinu enzima MMP-9 u urinu, uz povećanje stanica urođene imunosti u perifernoj krvi, što bi moglo doprinijeti praćenju, ranom otkrivanju bolesti i zaštiti te posljedičnom ranijem i učinkovitijem liječenju.