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IMMUNOTROPIC EFFECTS OF SO-CALLED SLAG METABOLITES (CREATININE, UREA, URIC ACID AND BILIRUBIN) AT RATS

Igor L. Popovych[1,3], Anatoliy I. Gozhenko[1], Igor L. Kuchma[1], Walery Zukow[2], Volodymyra R. Bilas[3], Galyna Y. Koval'chuk[4], Anzhela S. Ivasivka[4]

[1]Ukrainian Scientific Research Institute for Medicine of Transport, Odesa, Ukraine
prof.gozhenko@gmail.com; igorkuchma@ukr.net
[2]Nicolaus Copernicus University, Torun, Poland w.zukow@wp.pl
[3]Bohomolets' OO Institute of Physiology of National Academy of Sciences, Kyïv, Ukraine i.popovych@biph.kiev.ua
[4]Ivan Franko Pedagogical University, Drohobych, Ukraine
galynakovalchuk5@gmail.com

Abstract

Background. The study of the effect of end products of protein and nucleic acid metabolism on the immune system is of interest not only theoretical but also practical in line with the problems of urotoxins and hemodialysis. In previous studies, we found significant links between uricemia and uricosuria, on the one hand, and immune parameters, on the other hand. In this study, the spectrum of nitrogenous metabolites was expanded due to creatinine, urea and bilirubin. **Material and methods**. Experiment was performed on 60 healthy female Wistar rats. The plasma level and urinary excretion of the nitrogenous metabolites were determined. Immune status was assessed by thymocytogram, splenocytogram, blood leukocytogram and immunocytogram, as well as phagocytosis of blood neutrophils and monocytes. **Results**. Both negative and positive metabolic-immune correlations were revealed. Calculation of multiple correlation coefficients between individual metabolite parameters and constellations of immune parameters revealed the maximum immunotropic effect of uricosuria (R=0,637). This is followed by excretion of urea (R=0,617) and creatinine (R=0,606), bilirubinemia (R=0,589), creatinineemia (R=0,567), uricemia (R=0,566) and plasma urea level (R=0,500). The canonical correlation between the constellation of nitrogenous metabolites, on the one hand, and the parameters of immunity, on the other hand, was very strong: R=0,921; $\chi^2_{(154)}$=282; $p<10^{-6}$. **Conclusion**. Nitrogen metabolites exhibit significant immunotropic activity, both suppressor and enhancing.

Key words: uric acid, creatinine, urea, bilirubin, immunity, relationships, rats.

INTRODUCTION

The study of the effect of end products of protein and nucleic acid metabolism on the immune system is of interest not only theoretical but also practical in line with the problems of urotoxins and hemodialysis. In previous studies, we found significant links between uricemia and uricosuria, on the one hand, and immune parameters, on the other hand, in both healthy rats [17,18] and patients in Truskavets' spa [19,20,37]. In this experimental study, the spectrum of nitrogenous metabolites was expanded due to creatinine, urea and bilirubin.

MATERIAL AND METHODS

Experiment was performed on 60 healthy female Wistar rats 240-290 g. Of these, 10 remained intact, while others received drinking water of various compositions during the week. The day after the completion of the drinking course in all rats, at first, a sample of peripheral blood (by incision of the tip of the tail) was taken for leukocytogram analysis. Animals were then placed in individual chambers with perforated bottom for collecting daily urine. The experiment was completed by decapitation of rats in order to collect as much blood as possible.

The plasma level of the nitrogenous metabolites were determined: creatinine (by Jaffe's color reaction by Popper's method), urea (urease method by reaction with phenolhypochlorite), uric acid (uricase method) and bilirubin (by diazoreaction using the Jedrashik-Kleghorn-Grof method). The same metabolites, with the exception of bilirubin, were also determined in the daily urine. The analyzes were carried out according to the instructions described in the manual [15]. The analyzers "Pointe-180" ("Scientific", USA) and "Reflotron" (Boehringer Mannheim, BRD) were used with appropriate sets.

In the blood, the parameters of immunity were determined as described in the manual [31]: the relative content of the population of T-lymphocytes in a test of spontaneous rosette formation with erythrocytes of sheep by Jondal M et al [24], their theophylline-resistant (T-helper) and theophyllin-sensitive (T-cytolytic) subpopulations (by the test of sensitivity of rosette formation to theophylline by Limatibul S et al [26]; the population of B-lymphocytes by the test of complementary rosette formation with erythrocytes of sheep by Bianco C [5]. Natural killers were identified as large granules contain lymphocytes.

About the state of the phagocytic function of neutrophils (microphages) and monocytes (macrophages) were judged by the phagocytary index, the microbial count and the killing index for Staphylococcus aureus (ATCC N25423 F49) [6,11].

Immune organs weighed and made smears-imprints for counting splenocytogram and thymocytogram [3,4,6]. For them, as well as leukocytogram, CE Shannon's entropy was calculated [33].

Digital material is statistically processed on a computer using the software package "Statistica 20".

RESULTS AND DISCUSION

According to calculations by the formula:

$|r|=\{\exp[2t/(n - 1,5)^{0,5}] - 1\}/\{\exp[2t/(n - 1,5)^{0,5}] + 1\}$

for a sample of n=60 critical value |r| at $p<0,05$ ($t>2,00$) is 0,25, at $p<0,02$ ($t>2,39$) is 0,30, at $p<0,01$ ($t>2,66$) is 0,33, at $p<0,001$ ($t>3,46$) is 0,42.

Screening of linear correlation coefficients between parameters of nitric metabolites, on the one hand, and the recorded parameters of immunity, on the other hand, revealed the following (Table 1). The table does not include registered immunity parameters, which did not reveal any noteworthy correlation coefficient with nitrogenous metabolites. The general impression is that along with the expected negative correlations, there are positive correlations.

In the next step of the analysis, a regression model was constructed for each plasma and urine nitrogen metabolite by stepwise exclusion until the maximum level of adjusted R^2 was reached. As a result, it turned out that some regression models included parameters with an insignificant correlation coefficient, while some parameters with a significant correlation were outside the model.

Now let's analyze each regression model.

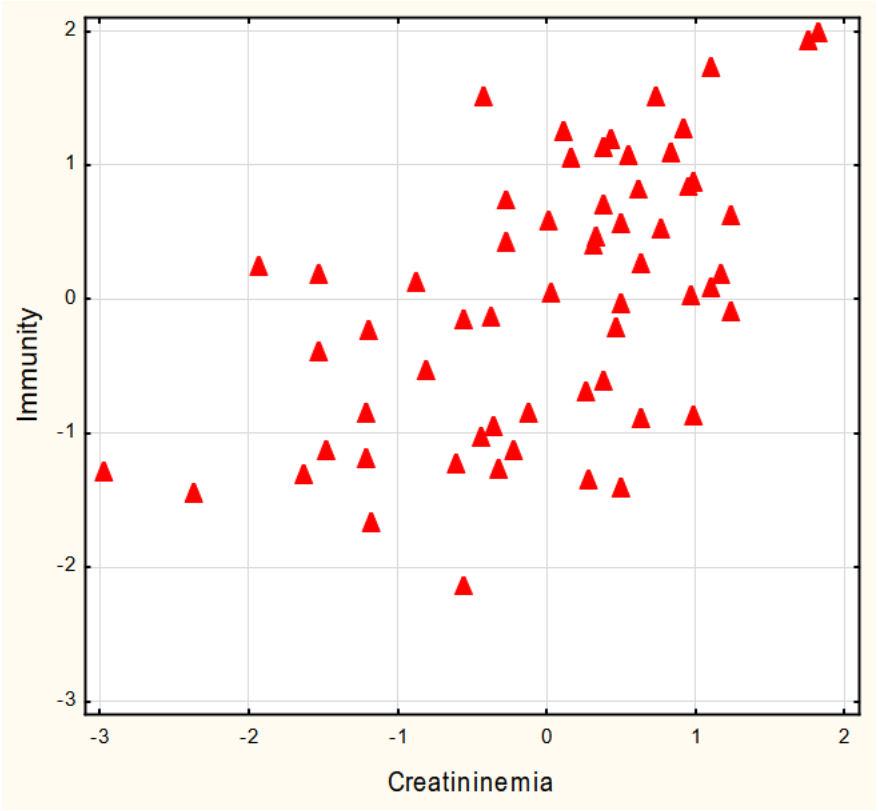
From the regression model it follows that creatinineemia upregulates the relative content in the blood of theophylline-resistant T-lymphocytes and the relative mass of the spleen, while downregulates the content of reticulocytes in the thymus and the entropy of the leukocytogram. Judging by adjusted R^2, the rate of immunomodulation is 27,2% (Table 2 and Fig. 1).

Table 1. Correlation matrix for metabolic and immune parameters

	Correlations						
Variables	CrEx	CrP	UreaEx	UAEx	Bilir	UreaP	UAP
Creatinine Excretion	1,00	0,04	0,59	0,39	0,04	0,14	0,15
Creatinine Plasma	0,04	1,00	0,08	0,13	0,25	0,84	-0,28
Urea Excretion	0,59	0,08	1,00	0,35	0,27	0,16	0,12
Uric Acid Excretion	0,39	0,13	0,35	1,00	0,13	0,06	0,48
Bilirubin Plasma	0,04	0,25	0,27	0,13	1,00	0,25	-0,16
Urea Plasma	0,14	0,84	0,16	0,06	0,25	1,00	-0,31
Uric Acid Plasma	0,15	-0,28	0,12	0,48	-0,16	-0,31	1,00
Leukocytogram Entropy	-0,06	-0,26	-0,17	0,00	-0,19	-0,19	0,24
Thymocytogram Entropy	-0,01	-0,06	-0,25	-0,25	-0,04	0,22	-0,19
Splenocytogram Entropy	0,08	0,23	0,12	-0,10	0,27	0,21	-0,15
Microbial Count Neutrophils	-0,23	0,14	0,07	0,53	0,28	-0,06	0,25
Phagocytic Index Monocytes	0,19	-0,17	-0,05	0,06	-0,09	-0,23	0,05
Phagocytic Index Neutrophils	-0,08	0,13	0,20	0,30	0,18	0,13	0,09
Spleen Mass Index	0,25	0,30	0,08	0,00	-0,10	0,24	-0,03
Lymphoblastes Spleen	-0,09	0,01	0,40	0,11	0,42	-0,03	0,05
Lymphocytes Spleen	-0,16	-0,23	-0,03	-0,02	-0,11	-0,28	0,12
Plasmocytes Spleen	-0,28	0,22	0,15	-0,01	0,42	-0,03	-0,11
Fibroblastes Spleen	0,40	0,15	0,14	0,20	-0,03	0,17	0,13
Microphages Spleen	-0,18	0,04	-0,22	-0,08	-0,22	0,08	-0,01
Eosinophiles Spleen	0,02	-0,05	-0,22	-0,35	-0,21	-0,05	-0,10
Thymus Mass Index	-0,03	0,09	-0,24	-0,05	-0,19	0,14	-0,13
Lymphocytes Thymus	0,03	0,12	0,34	0,29	0,11	-0,12	0,18
Lymphoblastes Thymus	-0,30	0,07	-0,04	0,00	0,14	-0,05	-0,19
Reticulocytes Thymus	0,06	-0,24	-0,11	-0,26	-0,16	-0,08	0,12
Epitheliocytes Thymus	-0,01	-0,20	-0,45	-0,29	-0,33	-0,12	-0,09
Endotheliocytes Thymus	0,01	0,13	0,01	0,08	0,19	0,27	0,04
Macrophages Thymus	0,24	0,07	0,31	0,18	0,14	0,21	-0,05
Hassal's corpuscles Thymus	0,07	0,08	-0,07	-0,09	0,10	0,26	-0,28
Leukocytes Blood	-0,30	-0,00	-0,14	-0,17	-0,06	-0,04	-0,07
Monocytes Blood	0,20	0,01	-0,13	-0,45	-0,30	0,07	-0,35
Pan-Lymphocytes Blood	-0,10	0,18	-0,10	0,30	-0,01	0,03	0,07
Stub Neutrophils Blood	-0,03	-0,02	0,06	-0,23	0,07	0,00	-0,14
Eosinophiles Blood	0,00	-0,08	0,04	-0,06	0,28	-0,01	0,05
Natural Killers Blood	0,13	-0,11	-0,16	-0,41	-0,30	-0,01	-0,23
T helper Lymphocytes Blood	-0,10	0,31	-0,09	0,06	0,02	0,24	-0,03
T cytolytic Lymphocytes Blood	0,01	-0,12	0,07	0,10	0,12	-0,13	-0,05
B Lymphocytes Blood	-0,19	0,06	-0,09	0,17	0,14	0,04	-0,04

Table 2. Regression Summary for Creatinineemia
R=0,567; R^2=0,322; Adjusted R^2=0,272; $F_{(4,6)}$=6,5; p=0,0002

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
Variables	r		Intercpt	0,033	0,049	0,67	0,505
Th Lymphocytes Blood, %	**0,31**	0,370	0,113	0,0033	0,0010	3,27	0,002
Spleen Mass Index, g/100g	**0,30**	0,304	0,111	0,154	0,056	2,74	0,008
Reticulocytes Thymus, %	-0,24	-0,265	0,115	-0,0074	0,0032	-2,30	0,025
Entropy Leukocytogram	**-0,26**	-0,212	0,113	-0,115	0,061	-1,88	0,065

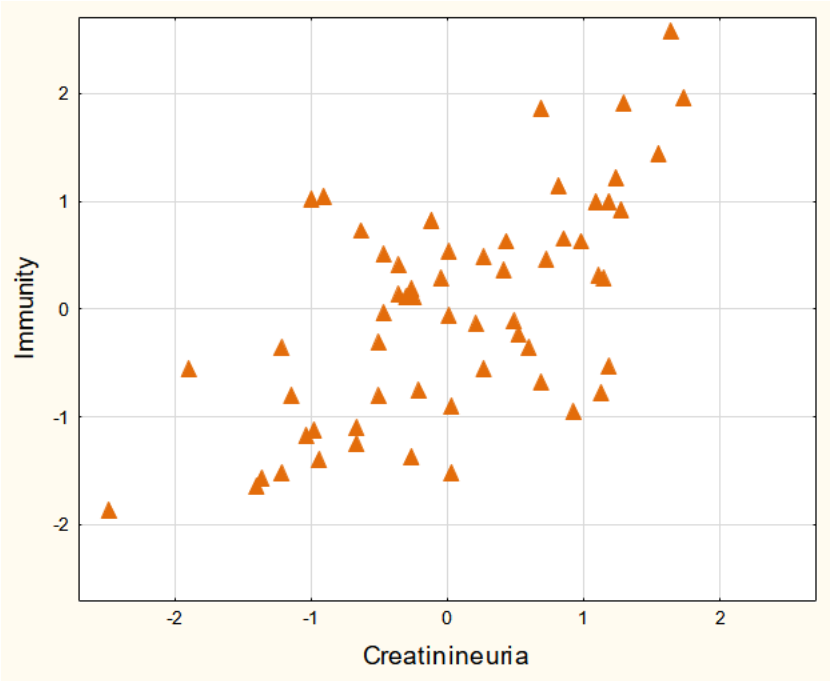


R=0,567; R^2=0,322; $\chi^2_{(4)}$=21,7; p=0,0002; Λ Prime=0,678
Fig. 1. Scatterplot of canonical correlation between Creatinineemia (X-line) and the Immunity (Y-line) in female rats

Creatinineuria is associated with another immune constellation. It upregulates the mass of the spleen and the content of fibroblasts in the splenocytogram, as well as the content of macrophages in the thymus, while downregulates the content in the thymus lymphoblasts, the level of leukocytes in the blood and the intensity of phagocytosis of microbes by blood neutrophils. The immunomodulatory ability of creatinineuria is 29,5% (Table 3 and Fig. 2).

Table 3. Regression Summary for Creatinineuria
R=0,606; R^2=0,367; Adjusted R^2=0,295; $F_{(6,5)}$=5,1; p=0,0003

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(53)}$	p-level
Variables	r		Intercpt	9,853	7,216	1,37	0,178
Fibroblastes Spleen, %	**0,40**	0,307	0,115	0,873	0,326	2,68	0,010
Spleen Mass Index, g/100g	**0,25**	0,200	0,118	14,56	8,578	1,70	0,096
Macrophages Thymus, %	0,24	0,200	0,122	0,859	0,521	1,65	0,105
Microbial Count Neutrophils	-0,23	-0,161	0,112	-0,578	0,401	-1,44	0,156
Leukocytes Blood, 10^9/L	**-0,30**	-0,215	0,116	-0,227	0,123	-1,86	0,069
Lymphoblastes Thymus, %	**-0,30**	-0,144	0,118	-0,705	0,576	-1,22	0,226



R=0,606; R^2=0,367; $\chi^2_{(6)}$=25,2; p=0,0003; Λ Prime=0,633
Fig. 2. Scatterplot of canonical correlation between Creatinineuria (X-line) and the Immunity (Y-line) in female rats

The correlation coefficients between plasma urea and immune parameters are on the verge of significance. As a result, the degree of their determination is only 18,1%, but statistically significant (Table 4 and Fig. 3).

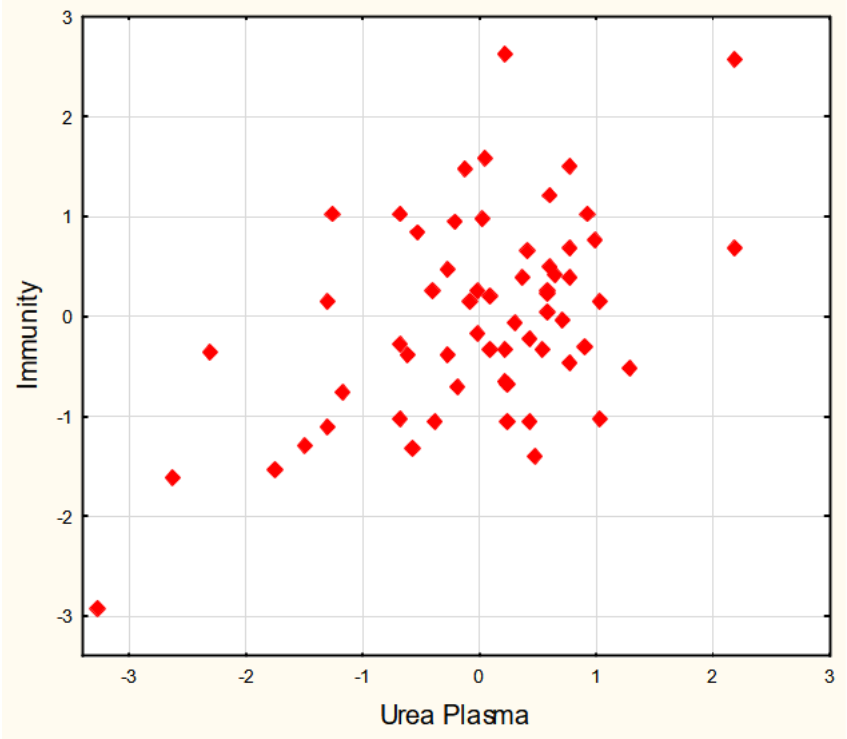
Table 4. Regression Summary for Urea Plasma
R=0,500; R^2=0,250; Adjusted R^2=0,181; $F_{(5,5)}$=3,6; p=0,007

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(54)}$	p-level
Variables	r		Intercpt	-3,254	4,339	-0,75	0,457
Endotheliocytes Thymus, %	**0,27**	0,223	0,124	0,733	0,409	1,79	0,079
Hassal's corpuscles Thymus, %	**0,26**	0,207	0,119	1,432	0,823	1,74	0,088
Spleen Mass Index, g/100g	0,24	0,173	0,121	8,007	5,607	1,43	0,159
Th Lymphocytes Blood, %	0,24	0,249	0,121	0,207	0,101	2,05	0,045
Phagocytic Index Monocytes, %	-0,23	-0,158	0,120	-0,573	0,437	-1,31	0,196

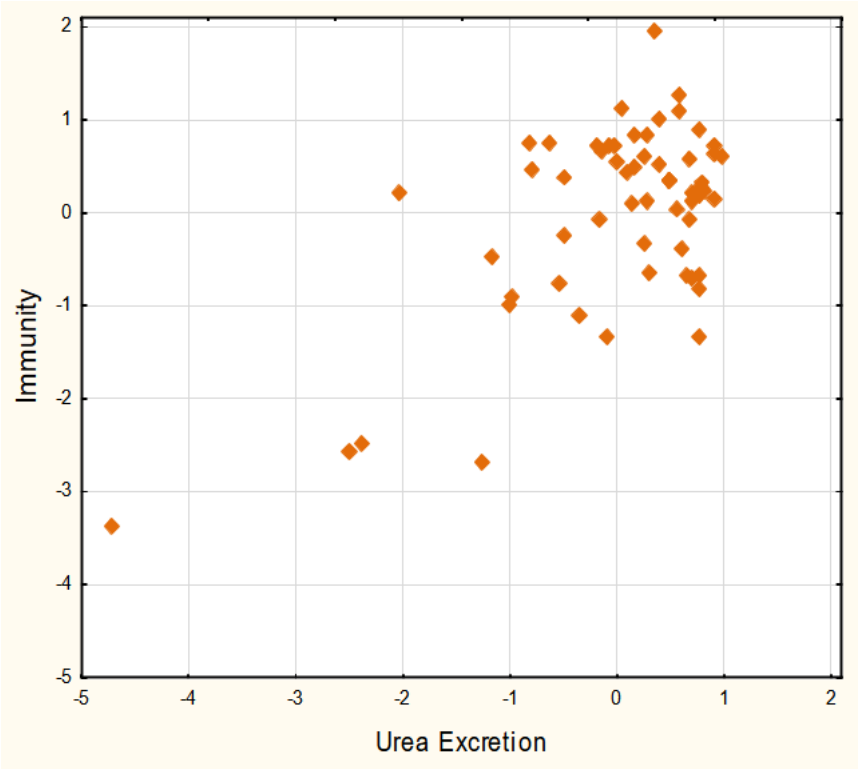
Instead, urea excretion bonds are stronger and reflect its enhancing effect on the content of lymphoblasts in the spleen as well as lymphocytes and macrophages in the thymus. The measure of positive determination is 34,7% (Table 5 and Fig. 4).

Table 5. Regression Summary for Urea Excretion
R=0,617; R^2=0,381; Adjusted R^2=0,347; $F_{(3,6)}$=11,5; p=10^{-5}

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(56)}$	p-level
Variables	r		Intercpt	-2137	565,5	-3,78	0,0004
Lymphoblastes Spleen, %	**0,40**	0,244	0,112	32,53	14,95	2,18	0,0338
Lymphocytes Thymus, %	**0,34**	0,430	0,119	29,13	8,08	3,61	0,0007
Macrophages Thymus, %	**0,31**	0,441	0,115	68,24	17,84	3,82	0,0003



R=0,500; R^2=0,250; $\chi^2_{(5)}$=16,0; p=0,007; Λ Prime=0,750
Fig. 3. Scatterplot of canonical correlation between Urea Plasma (X-line) and the Immunity (Y-line) in female rats



R=0,617; R^2=0,381; $\chi^2_{(3)}$=27,1; p=10^{-5}; Λ Prime=0,619

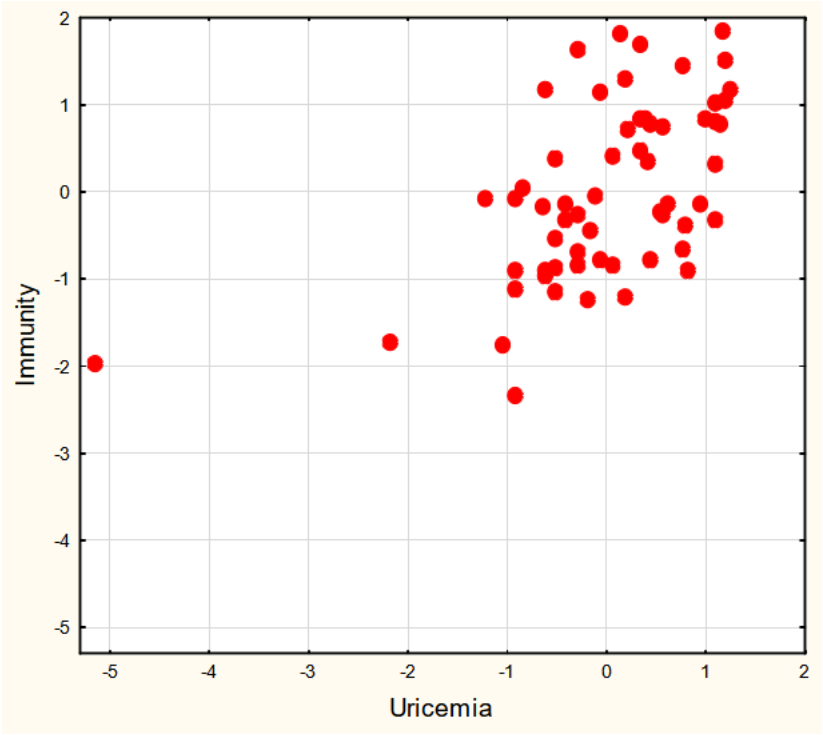
Fig. 4. Scatterplot of canonical correlation between Urea Excretion (X-line) and the Immunity (Y-line) in female rats

The immunotropic effect of uricemia is exclusively suppressive and is manifested in relation to the level in the blood of monocytes and natural killers and Hassal's corpuscles in the thymus, as well as the entropy of the blood leukocytogram. The measure of negative determination is 27,1% (Table 6 and Fig. 5).

Table 6. Regression Summary for Uricemia

R=0,566; R^2=0,320; Adjusted R^2=0,271; $F_{(4,6)}$=6,5; p=0,0002

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(55)}$	p-level
Variables	r		Intercpt	-1216	843	-1,44	0,155
Monocytes Blood, %	**-0,35**	-0,933	0,271	-171,2	49,7	-3,44	0,001
Hassal's corpuscles Thymus, %	**-0,28**	-0,250	0,113	-244,8	110,0	-2,23	0,030
Entropy Leukocytogram	-0,24	0,292	0,113	2044	788	2,59	0,012
Natural Killers Blood, %	-0,23	0,633	0,271	128,5	55,0	2,34	0,023



R=0,566; R^2=0,320; $\chi^2_{(4)}$=21,6; p=0,0002; Λ Prime=0,680

Fig. 5. Scatterplot of canonical correlation between Uricemia (X-line) and the Immunity (Y-line) in female rats

The immunotropic effects of uricosuria are ambiguous. It upregulates the intensity (Fig. 6) and activity of phagocytosis, while downregulates blood levels of eosinophils and rod-shaped neutrophils, as well as reticulocytes in the thymus. The degree of immunomodulation is 35,1% (Table 7 and Fig. 7).

Bilirubin upregulates the content in the splenocytogram of lymphoblasts and its entropy, as well as the level of eosinophils in the blood and the intensity of phagocytosis, while downregulates the content in the splenocytogram of microphages. The degree of immunomodulation is 28,6% (Table 8 and Fig. 8).

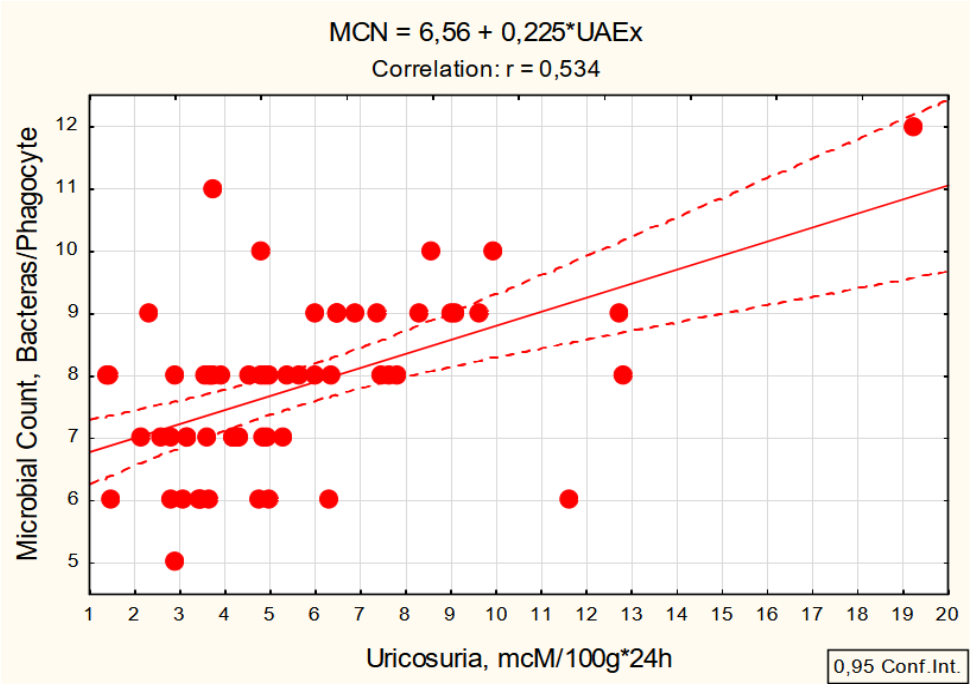


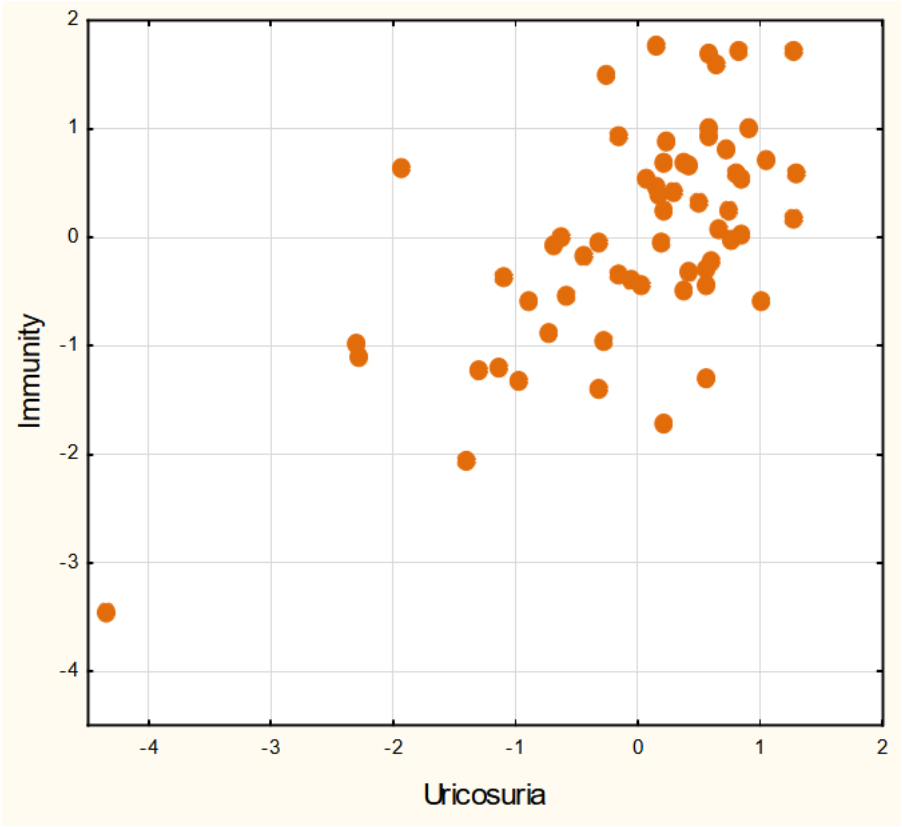
Fig. 6. Scatterplot of correlation between Uricosuria (X-line) and Microbial Count of Blood Neutrophils (Y-line) in female rats

Table 7. Regression Summary for Uricosuria
R=0,637; R^2=0,406; Adjusted R^2=0,351; $F_{(5,5)}$=7,4; $p<10^{-4}$

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(54)}$	p-level
Variables	r		Intercpt	10,09	7,64	1,32	0,192
Microbial Count Neutrophils	**0,53**	0,539	0,140	1,278	0,331	3,86	0,0003
Phagocytic Ind Neutrophils, %	**0,30**	-0,163	0,140	-0,139	0,119	-1,16	0,251
Stub Neutrophils Blood, %	-0,23	-0,204	0,111	-0,572	0,311	-1,84	0,071
Reticulocytes Thymus, %	**-0,26**	-0,128	0,113	-0,343	0,302	-1,14	0,261
Eosinophiles Spleen, %	**-0,35**	-0,247	0,110	-0,950	0,422	-2,25	0,029

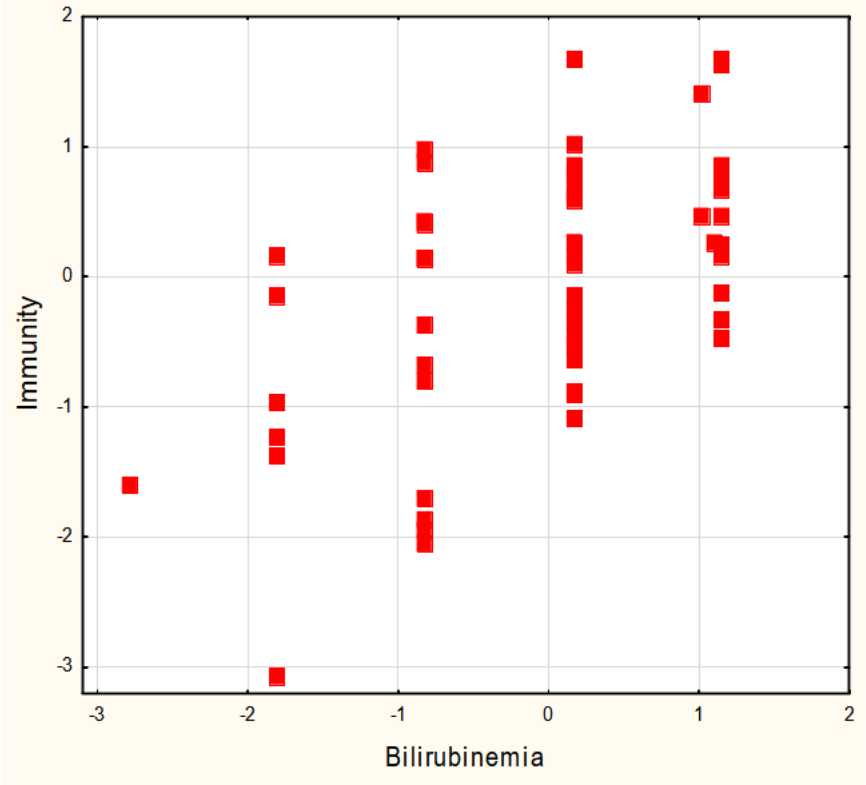
Table 8. Regression Summary for Bilirubinemia
R=0,589; R^2=0,346; Adjusted R^2=0,286; $F_{(5,5)}$=5,7; p=0,0003

		Beta	St. Err. of Beta	B	St. Err. of B	$t_{(54)}$	p-level
Variables	r		Intercpt	-8,139	8,457	-0,96	0,340
Lymphoblastes Spleen, %	**0,42**	0,329	0,119	0,540	0,195	2,76	0,008
Eosinophiles Blood, %	**0,28**	0,253	0,111	0,278	0,122	2,28	0,027
Microbial Count Neutrophils	**0,28**	0,203	0,113	0,323	0,180	1,79	0,079
Entropy Splenocytogram	**0,27**	0,133	0,116	12,82	11,18	1,15	0,257
Microphages Spleen, %	-0,22	-0,196	0,110	-0,206	0,116	-1,78	0,081



R=0,637; R^2=0,406; $\chi^2_{(5)}$=28,9; $p<10^{-4}$; Λ Prime=0,594

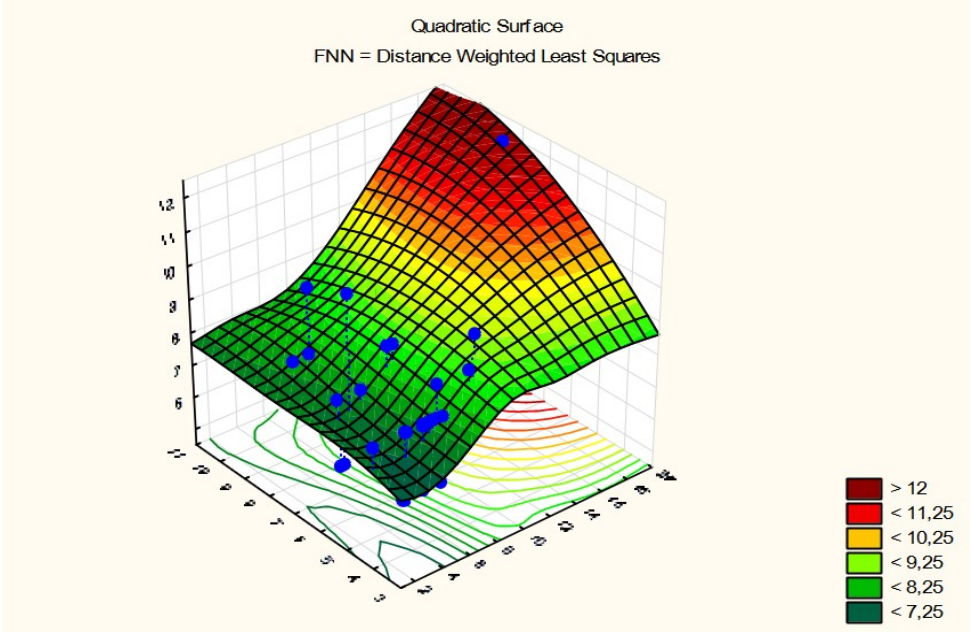
Fig. 7. Scatterplot of canonical correlation between Uricosuria (X-line) and the Immunity (Y-line) in female rats



R=0,589; R^2=0,346; $\chi^2_{(5)}$=23,6; p=0,0003; Λ Prime=0,654

Fig. 8. Scatterplot of canonical correlation between Bilirubinemia (X-line) and the Immunity (Y-line) in female rats

The combined effect of uricosuria and bilirubinemia on the intensity of neutrophil phagocytosis is only slightly greater than the effect of uricosuria itself (Fig. 9).



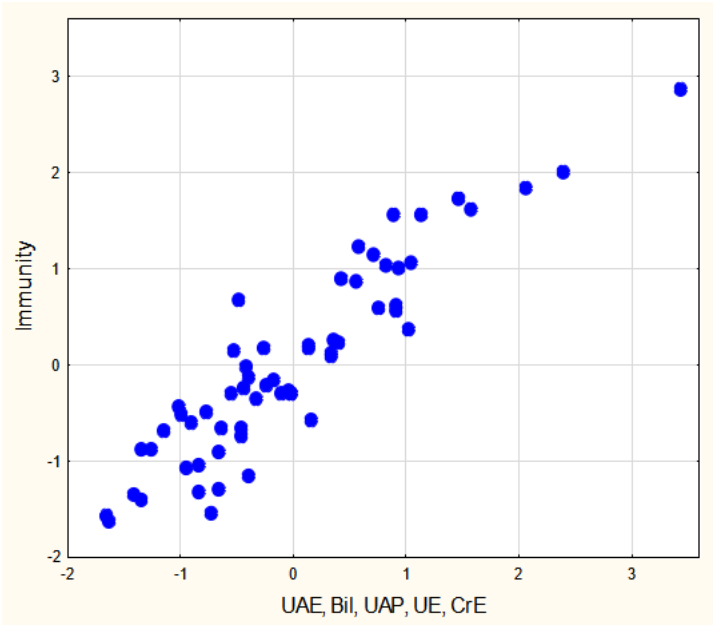
$Z=6,00+0,213•X+0,136•Y$; $R=0,575$; $R^2=0,331$; Adjusted $R^2=0,307$; $F_{(2,6)}=14$; $p<10^{-5}$

Fig. 9. The combined effect of uricosuria (µM/100g•24h; **X-line) and bilirubinemia (µM/L; Y-line) on the intensity of neutrophil phagocytosis (Bacteras/Phagocyte; Z-line)**

Because nitrogenous metabolites and immunocytes do not interact alone, but as biochemical and immune complexes, the method of choice for evaluating such interactions is canonical correlation (Table 9).

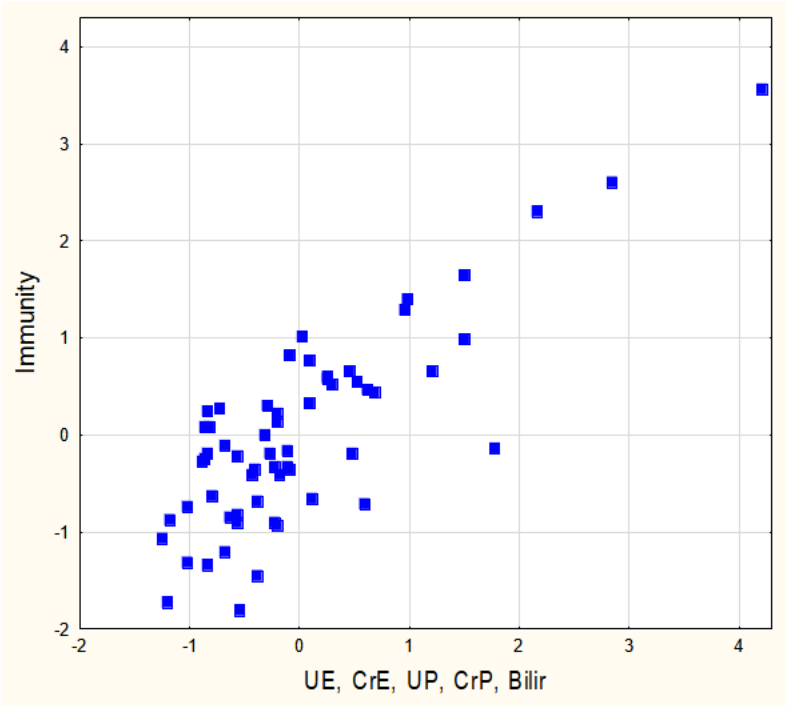
Table 9. Factor load on canonical roots of nitric metabolites and immunity parameters

Left set	Root 1	Root 2
Uricosuria	**,686**	,070
Bilirubinemia	**,438**	**,206**
Uricemia	**,392**	-,011
Urea Excretion	**,264**	**,897**
Creatininuria	**-,242**	**,511**
Urea Plasma	-,089	**,394**
Creatininemia	,036	**,348**
Right set	**Root 1**	**Root 2**
Microbial Count Neutrophils	**,803**	-,113
Lymphoblastes Spleen, %	**,479**	**,391**
Phagocytic Index Neutrophils, %	**,441**	,143
Lymphocytes Thymus, %	**,428**	**,347**
Lymphoblastes Thymus, %	**,272**	-,085
Endotheliocytes Thymus, %	**,091**	,022
Th Lymphocytes Blood, %	**,067**	-,012
Eosinophiles Blood, %	**,067**	,015
Monocytes Blood, %	**-,745**	,060
Natural Killers Blood, %	**-,644**	-,047
Eosinophiles Spleen, %	**-,468**	-,103
Reticulocytes Thymus, %	**-,345**	-,073
Hassal's corpuscles Thymus, %	**-,201**	-,063
Spleen Mass Index, g/100g	**-,250**	**,261**
Entropy Splenocytogram	-,053	**,246**
Macrophages Thymus, %	,082	**,305**
Fibroblastes Spleen, %	-,132	**,199**
Stub Neutrophils Blood, %	-,139	**,147**
Entropy Leukocytogram	-,026	**-,265**
Microphages Spleen, %	-,092	**-,203**
Phagocytic Index Monocytes, %	-,114	**-,141**
Leukocytes Blood, 10^9/L	,020	**-,113**



R=0,921; R^2=0,848; $\chi^2_{(154)}$=282; p=10^{-6}; Λ Prime=0,0032

Fig. 10. Scatterplot of canonical correlation between the nitric metabolites (X-line) and the Immunity (Y-line) in female rats. The first pair of roots



R=0,816; R^2=0,665; $\chi^2_{(126)}$=169; p=0,006; Λ Prime=0,021

Fig. 11. Scatterplot of canonical correlation between the nitric metabolites (X-line) and the Immunity (Y-line) in female rats. The second pair of roots

As a result of canonical analysis, two pairs of canonical roots were formed. Nitrogen root of the first pair, judging by the factor loads, represents mainly uric acid and bilirubin. The immune root of the first pair contains information about the parameters of the **thymus**, **spleen** and **blood**, which are subject to **stimulating** or **suppressive** effects of uric acid and bilirubin. The immunomodulatory effect of these nitrogenous metabolites, judging by the coefficient of determination, is very significant (Fig. 10).

The nitrogen root of the second pair represents mainly urea and creatinine. Their immunomodulatory effect is directed to another constellation of immune parameters and is much weaker (Fig. 11). However, there is approximately the same effect of both pairs of metabolites on the content of lymphoblasts in the spleen and lymphocytes in the thymus, while different effects on the relative mass of the spleen.

DISCUSSION

Assuming that the correlations reflect causal relationships, uric acid can be found to have significant immunomodulatory activity, upregulating the activity and intensity of phagocytosis Staph. aureus by blood neutrophils, instead downregulating the content of rod-shaped neutrophils, monocytes and natural killers in the blood. Our findings are consistent with data from Martínez-Reyes CP et al [27], which showed in vitro that incubation of human macrophages for 12 hours in the presence of increasing concentrations of uric acid (0,23; 0,45 and 0,9 mM/L, ie comparable to its level in plasma) dose-dependently increased their phagocytic activity, which was defined as the percentage of macrophages containing labeled Escherichia coli. This was accompanied by increased expression of TL4-receptors and increased production of TNF-α. A possible mechanism by which uric acid exerts a pro-inflammatory effect on human macrophages affects the anionic transporter of urate URAT1 in a dose-dependent manner. URAT1, in turn, may enhance NF-κB activation and lead to the production of proinflammatory cytokines in ways yet to be elucidated. In the same

experiment, uric acid simultaneously inhibited the expression of CX3CR1- and CCR2-receptors, which are involved in inhibiting the recruitment of monocytes from the blood to sites of inflammation, where these cells differentiate into macrophages. This agrees well with our other fact about the downregulation by uric acid levels in the blood of monocytes, apparently due to the activation of their translocation into tissues.

The downregulation of natural killer blood level found in our study is also consistent with the data of Gao L et al [13], that in human asymptomatic hyperuricemia is accompanied by low levels of NK cells in the blood. However, Carvalho LAC et al [7] during incubation with uric acid of neutrophil-like cells (HL-60) stated inhibition of their killing activity against Pseudomonas aeruginosa as well as decreasing the release of the inflammatory cytokines.

It is known that innate phagocytes, including dendritic cells, macrophages and neutrophils, can use TL-receptors to recognize uric acid, more precisely sodium urate crystals, as one of the pro-inflammatory endogenous signals secreted by dead or damaged cells. These molecular structures associated with damage can trigger a systemic inflammatory response similar to molecular patterns associated with pathogens [14].

In addition to TL-receptors, adenosine receptors may mediate the immunotropic effects of uric acid. Uric acid (**2,6,8**-tri**oxipurine**) is a structural homolog of adenosine [(2R,3R,4R,5R)-2-(6-amino**purine**-il)-5-(hydroximethyl) oxolan-3,4-diol)] as well as theophylline (**2,6**-di**oxi**-1,3-dimethyl**purine** or 1,3-dimethyl**xantine**) and caffeine (**2,6**-di**oxi**-1,3,7-trimethyl**purine** or 1,3,7-trimethyl**xantine**). It is known that the immunotropic effect of adenosine is realized through its receptors (A_1, A_{2A}, A_{2B}, A_3), which express virtually all populations of immunocytes: T, NK, B lymphocytes, macrophages, neutrophils, dendritic and endothelial cells [1,22,23,42]. Methylxanthines are nonselective antagonists of adenosine receptors, mainly A_{2A} and A_1 [8,28,29,30,35]. The facts obtained in our [19,20] previous human studies on the inversely relationship of uricemia (the level of which is comparable to the concentrations of adenosine and theophylline in immune tests in vitro) with a relative blood content of $CD4^+CD3^+$ T-helper cells in combination with the presence of directly connection with the content of $CD8^+CD3^+$ T-cytolytic cells indicate the similarity of the effects of uric acid with those of adenosine, ie activation of A_{2A} receptors.

The second strongest immunomodulator in this study was bilirubin, which upregulates the intensity of neutrophil phagocytosis, the level of eosinophils in the blood and lymphoblasts in the spleen, as well as the entropy of the splenocytogram, while downregulates the level of macrophage in the spleen.

Phelan D et al [32] demonstrated that the heme degradation products bilirubin and biliverdin are aryl hydrocarbon receptor (AhR) ligands which can regulate the AhR-dependent gene expression pathway. AhR translocates into the nucleus upon binding of various small molecules into the pocket of its single-ligand binding domain. AhR binding to both xenobiotic and endogenous ligands results in highly cell-specific transcriptome changes and in changes in cellular functions [12]. Although the AhR was initially recognized as the receptor mediating the pathologic effects of dioxins and other pollutants [2], the activation of AhR by endogenous and environmental factors has important physiologic effects, including the regulation of the immune response [9,10,36,43]. Both adaptive and innate immune cells require AhR signaling at critical checkpoints. AhR signaling is considered a promising drug and preventive target, particularly for cancer, inflammatory, and autoimmune diseases [12].

It is noteworthy that currently known receptors for the implementation of immunotropic effects are the most powerful among our studied - uric acid and bilirubin, while reports of immunocyte expression of receptors for creatinine and urea on the resources of PubMed and PMC we could not find, as well as work on immunotropic effects creatinine and urea.

Since they do occur, we hypothesize the realization of the immunotropic effects of these and previous nitrogenous metabolites through their effect on neurogenic and endocrine

mechanisms of immunomodulation. Our hypothesis is based on the concepts of functional-metabolic continuum [16] and neuroendocrine immunomodulation [21,25,34,38-41,44].

Data in support of our hypothesis will be given in subsequent publications.

CONFORMITY TO ETHICAL STANDARDS

Experiments on animals have been carried out in accordance with the provisions of the Helsinki Declaration of 1975, revised and supplemented in 2002 by the Directives of the National Committees for Ethics in Scientific Research.

The conduct of experiments was approved by the Ethics Committee of the Ukrainian Scientific Research Institute for Medicine of Transport. The modern rules for the maintenance and use of laboratory animals complying with the principles of the European Convention for the Protection of Vertebrate Animals used for scientific experiments and needs are observed (Strasbourg, 1985).

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