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Compensatory functions of suppressed immune system of the organism in experimental and clinical oncology: the impact of natural antibodies to endotoxin (review of a new conception and its methodological aspects)

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The aim of the review is to represent a new conception that the specific IgMNA to endotoxin influence the natural compensatory responses of a suppressed immune system. Many-year (since 1970) studies on natural endotoxin immunity, carried out at Preventive Immunology Laboratory of Vilnius University Institute of Oncology, have shown that the natural and acquired immune functions of both immunostimulated and immunosuppressed organisms (of experimental animals, practically healthy subjects and people professionally exposed to harmful factors, ill with oncological and other chronic diseases) are closely related to IgM class natural antibodies (NA) to the enterobacterial common antigen (ECA) present in the internal layer of the external membrane of intestinal microorganism cells, i.e. endogenous antibodies to enterobacterial lipopolysaccharid (endotoxin). With age, when cellular immunity functions deteriorate, the level of these NA to endotoxin in blood serum increases. It was stimulated or suppressed in experimental rats depending on the doses of cancinogenic substances and chemotherapeutic drugs. The review also shows that the NA to endoxin population level (PLNA%) is downregulated by tobacco smoking, alcohol consumption, living in an environment polluted by industrial exhausts, professional exposure to harmful agents, and chronic diseases. IgMNA to endotoxin represent the natural compensatory functions of a suppressed immune system. The complex of immune reactions related to endotoxin and specific IgMNA to endotoxin (PLNA%, phagocytosis, leukocyte migration inhibition reaction (LMIR), leukocyte adherence inhibition reaction (LAIR), total IgM and total IgG) reflects enhancement of the natural immune functions of the organism on the background of the suppressed functions of classical cellular immunity and suits well the purpose of elucidating the natural compensatory responses of immune homeostasis related to endotoxin immunity. Under a more pronounced suppression, some of these functions may be also suppressed. In pharynx and hypopharynx cancer patients, suppression of the immune system (reduced lymphocyte percentage, most of T and B system indices, blasttransformation ability of lymphocytes) was accompanied by partial suppression of endotoxin immunity compensatory reactions (PhI, LMIR, PLNA%, total IgM, total IgG). In non-oncological chronically ill patients and in chemical industry workers these compensatory reactions were unchanged or, more frequently, enhanced, although the classical indices of cellular immunity suppression in these subjects were close to those of oncological patients.

Key words: natural endotoxin immunity, cellular immunity, humoral immunity, specific IgMNA to endotoxin, compensatory immune functions, immunosuppression and immunostimulation, immune homeostasis

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INTRODUCTION

The natural resistance functions of immunocompetent cells (monocytes, macrophages of various tissues, natural killers, neutrophils and their predecessors) including natural antibodies are very important in maintaining bodily homeostasis under the effect of external and internal factors. These immunity systems evolutionarily are earlier than the acquired immunity systems; they respond to environmental changes and not require preliminary immunization. Natural antibodies are supposed to have been acquired by the organisms in the process of evolution; their phylogenetic origin and ontogenetic development are presumed [1–3]. Also, their individual resistance to damage by cancinogens and other factors as in the cases of infection are pointed out [2–4].

In the course of evolutionary transformations, the immune system has acquired components capable of responding adequately to a flow of the antigens threatening with change of the genetic stability of tissues. Thus, under the effect of various internal and external environmental factors, all carcinogenic agents included, immunosuppressants in particular, alongside the suppressive response of cellular immunity also endogenous compensatory reactions of immunostimulation are evoked [5–7].

Our previous studies have shown that data on the immune state of an individual are impossible to characterize exhaustively without data on the compensatory endogenous responses of such a mobile system as the immune one. Natural endotoxin immunity involves also immune compensatory functions [5–8].

The immune system without compensatory functions is unable to ensure the integrated immunoregulatory functions necessary for the organism's survival. It is important to establish the natural ability of healthy and sick persons to regulate the status of immune homeostasis. Thus, compensatory reactions are spontaneous and contribute to strengthening the immune safeguarding against the formation of endogenous risk factors of cancer and other diseases. They help maintain a balance that favours the organism's survival under the threat or in presence of immunosuppression. Together with immunosuppression, spontaneously the compensatory reactions arise, which represent the immunostimulatory variant arising together with immunosuppression under the effect of harmful factors. It is a known fact that the immune system is a protective system which helps the organism to survive in an unfavourable environment. The resistance of organisms and of individual cells to damage and their survival is the basic evolutionary law in organic nature [9, 10].

In our understanding, an essentially changed balance of the immune system, given the constantly changing interrelations of its numerous components, does not allow to reveal a pathology by merely measuring separate immunological indices; it requires an indispensable grouping of the interrelated components of the immune system. A methodical implementation of these theses was started by us in 1979 [5, 6].

Natural antibodies (NA) to enterobacterial common antigen (ECA, i.e. lipopolysaccharid – LPS) of Enterobacteriacea cells and other compensatory functions of immune homeostasis linked with this phenomenon counterbalance the suppressed functions of cellular immunity [11]. In this way the compensatory reactions of the immune system to the changing environment have helped mammalia, including man, to survive in the process of evolution.

The essence of this investigation of the compensatory functions of the immune system was the search of specific IgMNA to ECA from *B. faecalis alcaligenes 415* (the new denomination – *Alcaligenes faecalis 415*) (antibodies to endotoxin) and the estimation of their role in the natural compensatory mechanisms of immune state while determining age-related changes and in cases of diseases arising under suppressed immune functions.

The objective of the present study is to review the research work carried out by the Laboratory of Preventive Immunology on the spontaneous endotoxin immunity compensatory functions under immune imbalance (immunosuppression): cancer process, ageing, living in a contaminated environment, professional exposure to chemical substances, tobacco smoking, alcohol consumption, and, based on the summarized data, to propose an integrated approach to investigation of natural endotoxin immunity functions with the aim of defining the graveness of oncological diseases, and employing the natural endotoxin immunity reactions for prognosticating the outcome of chemotherapy, postoperative complications and mortality.

CHARACTERIZATION OF METHODOLOGICAL ASPECTS

In our studies we applied a complex of reactions related to endotoxin and specific IgMNA to endotoxin, searching for the phenomenon of compensatory immunostimulation. The methodology for investigation of natural endotoxin immunity was introduced as early as 1974 and approbated by the Soviet Science and Technology Committee (Moscow) [12]. Also, we applied a complex of reactions for investigating cellular immunity, in search of the immunosuppression phenomenon in order to elucidate the concomitant spontaneous immunostimulation.

Methods for obtaining endotoxin

In our studies, we used purified ECA (endotoxin) and crude extract of desintegrated cells of *Alcaligenes faecalis 415*. Purification included the following stages: 1) the growing of the biomass of the producer, 2) desintegration of cells and derivation of crude extract, 3) gel filtration, 4) ion exchange chromatography, 5) lyophilization [13, 14]. To prepare endotoxin, *Alcaligenes faecalis 415* was taken as a continuation of our previous work. For investigating the changeability of *E. coli*, faecal alkaline

producers were raised during antibiotic therapy. They are supposed to be the prototypes of all the Enterobacteriacea family, and the experiment presents one of the regeneration stages of the filtrated forms of these bacteria [15]. The strain of *Alcaligenes faecalis 415* was obtained from the Moscow Tarasevitch Medical Biological Standardization and Control Institute as a standard strain for our investigations [14]. The purified endotoxin preparation obtained from *Alcaligenes faecalis 415* preserved its full biological activity: antigenicy (precipitation and activity in ELISA) and immunogenicity in rabbits [14]. The NA to endotoxin detected in the healthy human blood belong to the IgM class.

Methods for detection of endogenous IgMNA to endotoxin

Rabbits were immunized with a crude extract of *Alcaligenes faecalis 415* (0.15–0.30 mg albumen and 0.2–0.3 ml physiological saline, intravenously; but only once) for the purpose of obtaining specific sera with IgMNA after 5–6 days. These sera gave only one precipitation band in gel-diffusion tests: the precipitation test and immunoautoradiography with purified endotoxin and *Alcaligenes faecalis 415* extract (Fig. 1), also with the control extracts of bacteria belonging to different Enterobacteriae species (Fig. 2) [13, 14, 16, 17]. In the test-systems, rabbit immune serum was used as a control serum. After five days the antibodies found in the serum belonged to IgM class [13].

Specific IgMNA to endotoxin were stated by the precipitation reaction according to Ouchterlony's method [18] modified by Gusev and Cvetkov [19]. We also introduced and applied the immunoautoradiographical methodology for stating NA to endotoxin, proposed by Elgort and Abelev [20] for determination of α-fetoprotein. These two methods of gel-diffusion were adapted for NA by Moncevičiūtė-Eringienė and are shown in Figs. 1 and 2. For stating the NA level, at the Laboratory of Preventive Immunology of Institute of Oncology R. Kemeklienė introduced the ELISA methodology [21], which according to its sensitivity equals the method of immunoautoradiography. This ELISA method was modified according to Lugowski and Romanowska's immunoenzymatic analysis method [22]. The modification is not yet finished.

Besides, to check the belonging of endotoxin natural antibodies to the IgM class of immunoglobulins, natural and immune sera were investigated employing Ouchterlony's method of precipitation in gel in reactions with commercial monospecific serum against total human IgM (159.5 mE/ml; 1.34 mg(ml) and with monospecific serum against total human IgG (124.5 mE/ml; 10.01 mg/ml) produced by the Gorki Scientific Research Institute of Epidemiology and Microbiology. The test sera in the reactions served as antigens and the total IgM and total IgG antiimmunoglobulin sera as antibodies (Fig. 3) [13, 23]. In blood serum, IgG class natural antibodies are usually found only in traces (22) or are completely absent in precipitation reactions in agar [13].

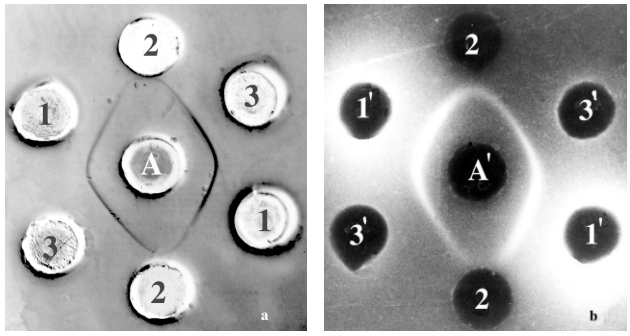

Fig. 1. Precipitation test (*a*) and immunoautoradiography (*b*) ECA (A) with immune specific rabbit serum to endotoxin–specific IgMNA (1) and human blood serum (3); 2 – physiological saline; A', 1', 3' – the same reaction components diluted 64 times

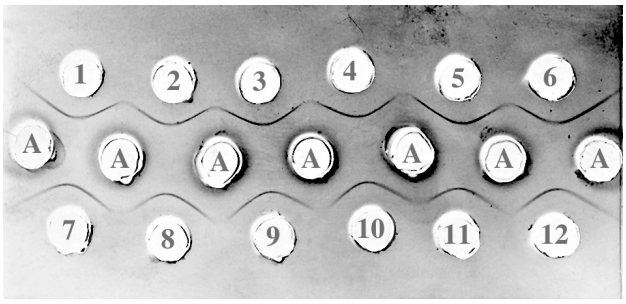

Fig. 2. Precipitation reaction in gel of extracts of *Alcaligenes faecalis 415* (1), *E. coli immobile Saccharosa* + (2), *E. coli immobile Lactosa* – (3), *E. coli* M17 (4), *E. coli alcalescens dispar* 02 (5), *Enterobacter aerogenes* (6) and cloacae (7), *Citrobacter* 01 (8), 018 (9) and 023 (10), *Proteus morganii* (11) and *vulgaris* (12) with specific rabbit antiserum (specific IgMNA) to endotoxin from *Alcaligenes faecalis 415* (A) (13)

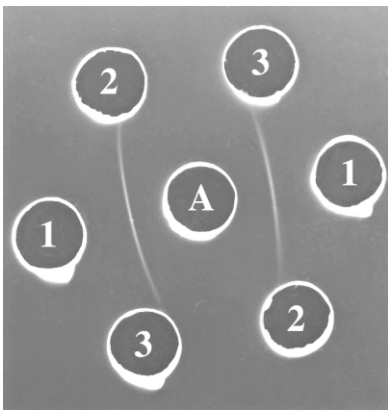

Fig. 3. Precipitation reaction in gel of rabbit serum specific to *Alcaligenes faecalis 415* obtained 5 days following single immunization (A) with monospecific sera to total human IgM (*1*) and total human IgG (*3*), 2 – physiological saline (13)

We determined the population level of NA to endotoxin (PLNA%) as a percentage of people in whose blood the mentioned antibodies were found.

Methods of determination of other spontaneous compensatory reactions of endotoxin immunity

Phagocytic activity of neutrophils (phagocytosis)

Phagocytic reaction indices: the phagocytosis number (PhN – the percentage of phagocytic neutrophils) and the phagocytosis index (PhI – the average number of bacteria phagocytized by one neutrophil) are determined by Specivtseva's method [24] modified by Nogachevski [25]. As the phagocytosis object, a daily culture of *Alcaligenes faecalis 415* bacteria from the family Enterobacteriaceae was used: 1 ml of physiological saline contains 1.8–2 billions of alive cells. Morphologically, these bacteria are short bacilli similar to cocci and thus easily countable.

Sensibilization of leukocytes to endotoxin was assessed by two methods: leukocyte migration inhibition (LMIR) and leukocyte adherence inhibition (LAIR) reactions.

Leukocyte migration inhibition reaction was carried out by Harrington and Stastny' micromodification in agarosis [26]. Two test samples are performed to each study individual. We used 100%, 50%, 25% ECA concentrations (in 100% ECA extract total protein concentration was 2.5 mg/ml). The migration index (MI) was assessed according to the formula:

$$MI = \frac{\text{average migration area with antigen}}{\text{average migration area without antigen}} \cdot 100\% .$$

Leukocyte adherence inhibition reaction was carried out according to Holan et al. [27]. Adherence of cells (AC) was expressed in percentage. The percentage of adhered cells was calculated after subtracting from 100% of cells the percentage of nonadhered cells, with calculating the arithmetical means of AC for each concentration of the antigen. The following concentrations were used: 0, 25, 50 and 100%.

Phagocytosis and both reactions for sensibilization of leukocytes to endotoxin were applied to *Alcaligenes faecalis 415* and endotoxin by Kazbarienė [28, 29].

Ascertainment of immunoglobulins. Total IgM, IgG and IgA concentrations were ascertained by the immunodiffusion method proposed by Mancini et al. [30].

Cellular immunosuppression determination methods

Leukocyte and lymphocyte counts were established using the conventional methods used at that time in clinical laboratory practice, such as leukocyte count determination in Goryayev's camera and with a haemocytometer, and lymphocyte count determination in a smear for counting the leukocyte formula and with a haemocytometer.

Lymphocyte population (total T-lymphocyte population $CD3^+$, $CD5^+$, helper/inductor subpopulation $CD4^+$, suppressor/cytotoxic lymphocyte subpopulation $CD8^+$, B lymphocytes $CD72^+$, natural killers $CD16^+$) was determined using monoclonal antibodies by the indirect immunofluorescence method [31]. Monoclonal antibodies were received from the Moscow Institute of Immunology company "Sorbent" (Russia).

The blasttransformation reaction was carried out according to Schütt's method [32], cultivating lymphocyte culture with phytohaemagglutinin (Wellcome, U. K.), and morphologically evaluated by Tesenov's method [33].

Data analysis was performed using an IBM < XT personal computer and the STATGRAPHICS version 2.0 system. Parametrical and nonparametrical methods were employed to verify the hypotheses. Hypotheses of the possibility of adverse results were used with the $p \le 0.05$ probability. The differences between the compared values and the probability $0.05 < p \le 0.1$ were regarded as tendencies to changes of the corresponding results [34].

NATURAL ENDOTOXIN IMMUNITY IN EXPERIMENTAL ONCOLOGY

With age, the specific IgMNA to endotoxin level in normal animal blood serum increases. A larger increase was noted in the bigger number of old rats as compared to young ones [23]. In 1970–74, investigations with carcinogens were carried out. NA to endotoxin were found in the sera of treated young Wistar rats in 60–70% of cases following subcutaneous administration of dimethylbenz(a)anthracene (DMBA) (4 mg/rat) or benzo(a)pyrene (BP) (5 mg/rat). Antibodies were revealed in 15–20% of normal age-matched rats ($p < 0.05$). It was assumed that activation of synthesis of natural antibodies could be one of the possible reasons for this phenomenon [3, 23].

In a later study, it was established that treating rats with DMBA, BP or methylcholantrene (MCH), the sera of 108 (58.7 ± 3.6%) from 184 treated and 15 (20.8 ± 4.8%) of 72 untreated rats reacted positively ($p < 0.05$) with extracts of different strains of *E. coli* and *Alcaligenes faecalis 415*. The antibodies to endotoxin showed no significant effect on the development of induced tumours in the same animals for 6 months. However, the activation of synthesis of the antibodies to endotoxin before injection of carcinogenic substances or of sera containing the antibodies during initial stages of the carcinogenic process markedly inhibited the development of the process, and the growth of induced tumours was slower [23, 35].

On injecting to Wistar rats immunosuppressants such as chemical carcinogenic substances (benzo(a)pyrene, methylcholantrene, N-nitrozodiethyamine) or active antitumoural preparations (vinblastin, 5-floruracyl, sarcolysin, 6-mercaptopurin, cyclophosphamide, methotrexate, lophenalium), thrice through 30 days primary immunostimulation was stated; later on we observed immunosuppression and secondary immunostimulation when the IgMNA to endotoxin test was used (Fig. 4) [23].

In trials with mongrel white rats, it was established that specific IgMNA synthesis was strongly activated in 2-month-old rats after intraperitoneal immunization with an extract of transjected rat tumours, both native and treated *in vitro* with alkylating preparations in doses destroying their grafting properties within 1 hour *in vitro*. NA were found in 91% of cases (Table 1) [23]. No changes in IgMNA synthesis were observed after immunization of rats with a normal rat tissue extract or with

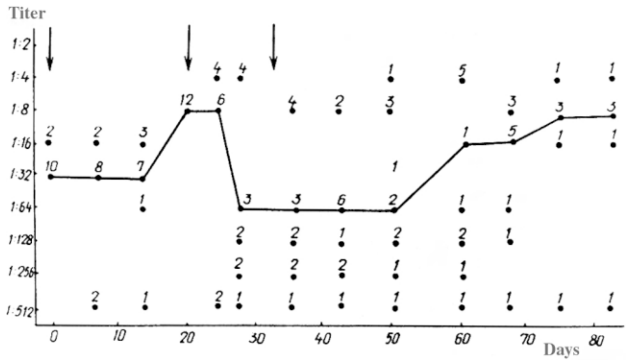

Fig. 4. Changes of specific IgMNA to endotoxin concentrations after threefold introduction of MCH in the organism. The arrows show the doses of MCH (5, 5 and 10 mg subcutaneously for one rat). Numerals above the points signify the number of synonymous dimensions (23)

extracts of the same tissues treated *in vitro* with alkylating preparations, after a triple administration of a 1% starch paste or alkylating preparations in very low doses, such as lophenalium synthesized at our Institute of Oncology (0.005–0.010 mg) or chlorambucil (0.002–0.004 mg) in 0.5 ml starch paste *per os* three times for one rat, with a week's interval. IgMNA were found only in 9% of rats (Table 1). Thus, according to our investigation data, extracts of transjected rat tumour tissues, both natural and treated with alkylating preparations, stimulate specific IgMNA to endotoxin synthesis [23].

Table 1. **Specific IgMNA to endotoxin in rats immunized with transjected tumour extracts only or treated with alkylating preparations *in vitro* (23)**

Test variant	Number of sera tests	Of them containing NA to endotoxin, %	
		n	% ± m%
Control groups			
Starch	118	8	7 ± 2.3
Minor doses of preparations	102	5	5 ± 2.1
Normal tissues	217	18	8 ± 1.9
Normal tissues + preparations	218	26	12 ± 2.2
Total	655	57	9 ± 1.1
Test groups			
Sarcoma 45	64	61	95 ± 2.7*
Sarcoma 45 + preparations	104	91	88 ± 1.2*
Sarcoma M–1	10	9	90 ± 10.6*
Jensen's carcinoma	9	8	89 ± 11.1*
Geren's carcinoma	10	10	100 ± 0.0*
Total	197	179	91 ± 2.0*

* p value < 0.05.

NATURAL ENDOTOXIN IMMUNITY IN HEALTHY HUMAN POPULATION

In 1979–87, 2570 practically healthy humans were investigated, among them 200 Vilnius city and district inhabitants (non-donors), 1335 Vilnius blood donors and 1035 Kaunas blood donors [36–39]. In 1988–91, 360 humans, among them working under harmful conditions, oncological and other patients [40–42], and in 1993–97 the immune state of 539 practically healthy humans [43–49] living in a district contaminated with industrial siftings (Trakai) and in a relatively less contaminated control district (Širvintos) were examined. The age of the majority of humans was 20–64 years. We divided them into three age groups: 20–34, 35–49 and 50–64 years.

In the first series (1979), specific IgMNA to endotoxin only was investigated in the blood serum of practically healthy 1030 humans according to age, sex, harmful work conditions and place of residence. Of them, 67% had natural antibodies. Besides, 32 individuals aged 18–19 years took part in the study, of them 62% had IgMNA to endotoxin (here, no comparison with other age groups was done) [36, 37].

Comparing all age groups over 35 (in this investigation there were three of them: 35–49 years, n = 380; 50–64 years, n = 189 and 65–79 years, n = 26) with the 20–34 age group (n = 403), the PLNA% (population level of natural antibodies in percentage) was higher by 10%, 18%, and 40%, respectively ($p \leq 0.05$).

In males under 50 years, natural antibodies were by 14% rarer than in females of the same age. Fourty per cent of males worked under partially harmful conditions, *vs*. only 1.5% of females in all age groups. The IgMNA index of 60% in males working under unharmful conditions was the same as that in females. In the 50–64 age groups of both males and females, the PLNA reached 78% [36].

More than 90% of humans involved in our study lived in towns and thus the first series yielded the decisive results. The rural females' PLNA% depended on age and increased to the same level as that of urban females. There were only separate individuals among the investigated males that lived in the country. Therefore their results were not considered [36].

The immune functions in relation to age and sex

More than 3000 inhabitants of the Vilnius and Kaunas cities and zones were investigated for specific IgMNA to endotoxin population levels (PLNA%) and changes in the leukocyte and lymphocyte indices in peripherial blood related with age. It was found that the level of IgMNA against endotoxin in blood serum and PLNA% increased and the quantity of leukocytes and lymphocytes decreased with age (Table 2) [36, 38, 39].

In the donor population older than 35, by 8% lower PLNA and by 5–6% higher leukocyte and lymphocyte levels were ascertained. The indices distributed according to sex proved that there were by 14–21% less males

Table 2. **Natural endotoxin immunity of Vilnius city inhabitants according to ANALL test-system (36)**

Age groups (years)	n	IgMNA (M ± m%) PLNA%	Leukocytes ($n \cdot 10^9$/l)	Lymphocytes ($n \cdot 10^9$/l)
20–34	280	48 ± 3.0	6.4 ± 0.13	1.8 ± 0.05
35–49	100	47 ± 4.9	6.1 ± 0.15	1.7 ± 0.02
50–64	200	74 ± 3.1*	5.7 ± 0.16*	1.6 ± 0.07*

* p value < 0.05.

aged under 50 than females of the same age having IgMNA in blood serum [36, 39].

In the male population, the leukocyte and lymphocyte count was in most cases higher than in females, but it did not change depending on age. However, the T lymphocyte number in peripheral blood increased with age. In the age group of 20–34 years it made 66 ± 1.7%, in the 35–49 year group 69 ± 1.0%, and in the 50–64 year group 72 ± 1.9%. Female total IgG and especially total IgM concentrations were higher than those of males. The total IgM concentration increased on decreasing the leukocyte and lymphocyte number [36, 39].

The ANALL test-system. The specific IgMNA to endotoxin test confirmed formation of immunosuppressive and immunostimulation states with reference to the individual's age. However, applying one test only, it was impossible to state the natural immunocompetence of the organism. IgMNA represented only the chain of humoral immunity. Therefore, firstly cellular elements (leukocyte and lymphocyte indices) were included into the investigation program, and the test-system "age-NA-leukocytes, lymphocytes" (ANALL) was recommended for a rapid estimation of the organism's endotoxin immune state. The test-system ANALL was suggested as an express methodology for a rapid definition of endotoxin immunity and as a methodology for introduction into practice the 6-type classification of human immune state [8, 16, 39]. Thus, the present work offers new variants of the former classical methods of determining the natural immune competence of the organism in dependence on the relations among immune reactions newly established with the aid of endotoxin and specific IgMNA to endotoxin.

For the control of the ANALL test-system, part of the same subjects (370 blood donors and 960 non-donors) were examined for the following cellular immunity indices of possible immunosuppression: $CD5^+$, $CD4^+$, $CD4^+/CD8^+$, $CD72^+$, $CD16^+$, blasttransformation reaction, LMIR, LAIR, phagocytosis and endotoxin or *Alcaligenes faecalis 415* as a substrate of these compensatory reactions were used to evaluate the cellular chain of endotoxin immunity. For the evaluation of the humoral chain of this immunity, the following indices were examined: total IgG, IgM and IgA concentration in the blood serum of 1840 humans [11, 38, 50].

While investigating the immunity parameters, it was stated that the ANALL system indices fairly accurately defined the compensatorily increased sensitivity of leukocytes to endotoxin, (LMIR, LAIR), elevations of PhN, PhI, total IgG, and IgM concentrations [28, 38, 40, 50].

The immune functions in relation to place of residence

In the city of Vilnius, in 1984–87 the increased quantity of pollutants caused by the growth of transport and factory emissions could be also related to launching a powerful thermal station. During this period investigations were carried out two times with a 2-year interval, (1984–85, n = 189; 1986–1987, n = 204) and the following lower indices were stated: the absolute number of leukocytes (10%), PLNA (14%) total IgG concentration (10%), total IgM concentration (30%) [39, 41].

In addition, comparative immunological investigations included 283 inhabitants in the Trakai district (contaminated by industrial siftings) and 256 inhabitants in the Širvintos district (less contaminated; control) in relation to factors of environmental pollution. The investigations were made when environmental pollution was decreasing (1993–94) and the common environmental emission of pollutants made only 50–60% of the 1989–1991 level [51]. It was confirmed that the immune state of young people (under 35) was stimulated and that of the older ones (over 35 years) was suppressed [43–45]. Further on, when pollution was still decreasing (investigations of 1995), the immune state of both young and older persons was found normalized [46]. On starting the use of orimulsion fuel in the Elektrėnai state power station, i.e. when the environmental pollution in the Trakai district increased [52], immunosuppression in both younger and older inhabitants was noted [47–49].

All these investigations proved an influence of age and environmental pollution factors on immune functions. In repeated investigations of 138 Vilnius inhabitants the majority of cellular immunity indices decreased and those of humoral immunity indices decreased or did not change [40–42]. The compensatory reactions of immune state were differentiated, and a methodology was suggested for defining them [40, 41]. On decreasing the environmental pollution by siftings in the Trakai district in 1993–94, prominent restoring compensatory reactions were noted [43].

Under the activity of environmental pollution factors, due to the use of orimulsion fuel by the Elektrėnai state electric power station, classic immunological indices were suppressed with reference to age both in common groups and in separate male and female groups (essentially smaller were leukocytes and lymphocytes, $CD5^+$, $CD4^+$, $CD16^+$ numbers, etc.). PLNA% did not differ and showed a compensatory reaction [47–49].

The immune functions with reference to harmful factors in chemical industry

The influence of the environment contaminated by industrial siftings on immune response was confirmed by the suppressed immune state among chemical industry workers [38, 39, 41].

In 1985, the immunological state of 60 individuals professionally exposed to chemical substances (aceton, phenol, formaldehyde, xylol, butylacetate, styrol, benzol, phthalic acid anhydride, ammonia, chromium, nickel, sulphuric, nitric and hydrochloric acids, etc.) was investigated. An analogous investigation after four years (1989) of the same workers showed that almost all immunological indices were statistically reliably lowered: total leukocyte and T lymphocyte counts were reduced by 20% and 4%, respectively, lymphocyte bllasttransformation reaction was weakened (blast number lowered by 24%). PLNA% was not changed, but some other compensatory reactions were lowered, among them neutrophil phagocytosis function (PhN and PhI were less by 12% and 31% and total IgM and IgG levels in blood serum by 26% and 16% respectively ($p \leq 0.05$) [41, 42].

It should be noted that specific IgMNA to endotoxin synthesis was suppressed by harmful work conditions (Fig. 5) [28], proving formation of the immunosuppressive mechanism in males professionally exposed to harmful factors.

Besides, the obtained results as well as the age-related changes allowed to suppose that, apart from industrial factors, the classical cellular immune functions and compensatory endotoxin immunity were influenced, more in males than in females, by negative habitual factors.

Immunomodulating effects of tobacco smoking and alcohol consumption

Tobacco smoking and alcohol abuse were found to increase the leukocyte and lymphocyte counts in peripheral blood and to decrease PLNA%. Statistically significant differences were noted, independently of sex, among labourers working under harmful and normal conditions and among individuals living in districts contaminated and less contaminated by industrial siftings. In subjects smoking 15–20 cigarettes a day, leukocyte count was higher by up to 17%, PLNA was lower by 24% (Fig. 6), and blood serum total IgM levels lower by 31% as compared to analogous indices in non-smokers [39, 53–55]. Tobacco smoking and alcohol consumption break the balance of the compensatory homeostatic response of LAIR and FAN indices, which depend on the presence or absence of specific IgMNA to endotoxin in blood serum [28]. Dispersion analysis revealed a relationship in the group of smokers between IgM concentration (g/l) and leukocyte indices ($<5.10^9$/l, 5–8 · 10^9/l, >8 · 10^9/l; F = 2.6; p = 0.07) [39]. In the donor group investigated in 1988–1990 there were 81% of males, of them 61% were smokers. Dispersion analysis demonstrated the influence of smoking on the total leukocyte count of donors: F = 2.7; p = 0.07 [42]. A particular emphasis should be put on PLNA in tobacco smokers aged 20–34 years: their PLNA was suppressed by 21% [37, 54].

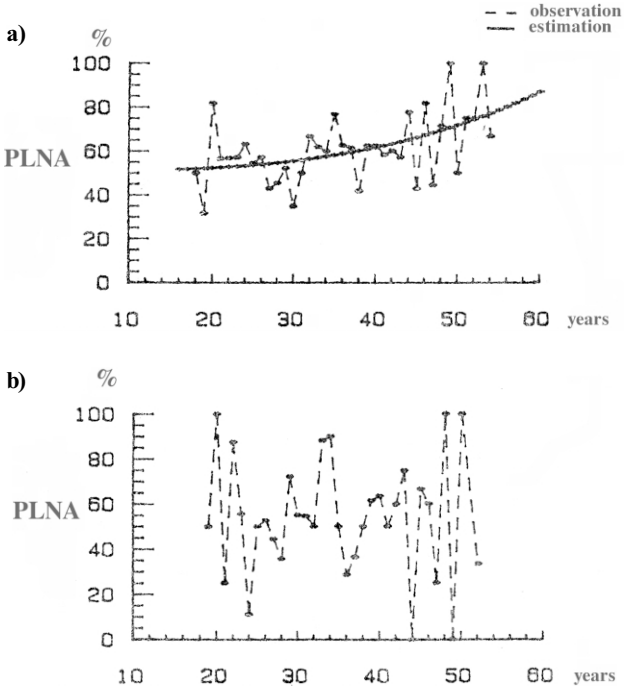

Fig. 5. Dependence of PLNA% on the age and harmfulness of work:
a) harmless working conditions (a clear increasing tendency);
b) harmful working conditions (no increasing tendency) (28)

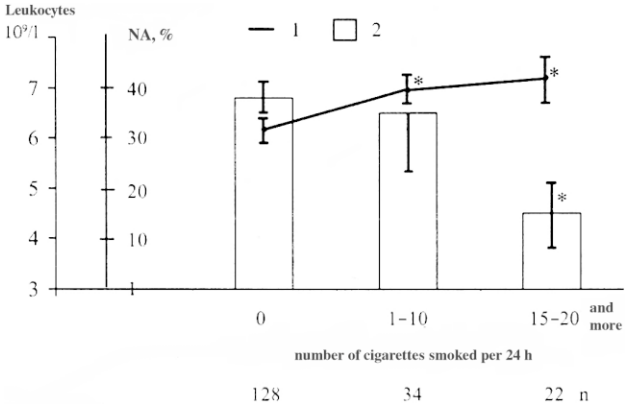

Fig. 6. The number of leukocytes (*1*) in peripheral blood of humans and PLNA% (*2*) depending on the number of smoked cigarettes per day; *p value < 0.05 (39).

NATURAL COMPENSATORY IMMUNE FUNCTIONS OF ENDOTOXIN IMMUNITY IN CANCER PATIENTS

Immune competence to endotoxin was investigated in 85 patients with larynx and hypopharynx cancer (histological diagnosis: Ca epidermoides) admitted to the Vilnius University Institute of Oncology clinic and not yet treated. The patients' age was 35–85 years. Their immune state was compared to the immune state of three control groups: blood donors (workers in harmless conditions), chemical industry workers and chronic non-oncological patients. Blood donors from the control group were aged

only under 65. Therefore, the immune state of oncological patients aged 35–65 years (n = 65) was compared first with that of blood donors aged 35–65 years (n = 57) and then with that of the age-matched humans professionally exposed to harmful factors (n = 60). The second group of oncological patients contained subjects aged 50–85 years (n = 62); it repeatedly included also part of patients from group 1 aged 50–65 to have the age group of oncological patients similar to that of the control group of chronically ill patients (n = 52).

The results showed that in pharynx and hypopharynx cancer patients aged 35–65 years immuncompetence was suppressed in comparison with that of age-matched donors; for instance, their lymphocyte count was by 11% lower ($p < 0.05$); the indices of the total subpopulation of T-lymphocytes, $CD4^+/CD8^+$ ratio and lymphocyte blasttransformation ability were also statistically significantly lower (by 12%, 29% and 10%, respectively, $p < 0.05$). Also, a tendency to lowered indices of the helpers/inductors ($CD4^+$) and B-lymphocyte subpopulations was noted [40, 42].

However, the decelerated type hypersensitivity index was elevated: leukocyte sensibilization to endotoxin in LMIR was accelerated. For instance, at 50% of endotoxin concentration the migration index (MI) in the patients was lower by 30% ($p < 0.05$), i.e. leukocyte sensibilization was more pronounced. Blood serum total IgA level was compensatorily elevated by 27% ($p < 0.05$). The PhI had a diminishing tendency, but the PhN was the same as in healthy humans [40, 42].

PLNA% in oncological patients, studied independently of age as a per cent index requiring a high number of observations, did not differ from control, but its numerical value was lower. Besides, in the group of pharynx and hypopharynx cancer patients aged 50–65 years, PLNA% was statistically significantly suppressed (by 39%) as compared to the same index in age-matched donors ($p < 0.05$). Thus, in the patients the synthesis of specific IgMNA to endotoxin was suppressed [40–42].

A comparison of cellular immunity indices for chemical industry workers and for donors revealed less differences than an analogous comparison for oncological patients and donors. In workers, three indices were lowered statistically significantly: lymphocyte $CD4^+$ helper/inductor subpopulation percentage, $CD4^+/CD8^+$ ratio and the blasttransformation ability of lymphocytes (lower by 20%, 19% and 16%, respectively; $p < 0.05$). Thus, immunosuppression in chemical industry workers was less pronounced than in pharynx and hypopharynx cancer patients, while compensatory immunostimulation was enhanced in the former. Even two LMIR indices indicated an upregulated leukocyte sensitivity to endotoxin. For instance, at a 100% antigen concentration the MI was less by 42% and at 50% by 27% ($p < 0.05$), i.e. leukocyte sensibilization was more pronounced. Blood serum total IgG was higher by 41% and total IgM by 54% ($p < 0.05$). The PLNA% and phagocytosis indices remained the same in both groups. However, PLNA% in chemical workers aged 50–65 years had a tendency to suppression as compared to the same index in donors of the same age [40–42].

A comparison of data on chemical workers aged 35–65 years and chronically ill patients aged 50–85 years (15–20 years older) showed similar suppressed and compensatory immune functions of the organism [42].

While comparing the same data for oncological patients aged 35–65 years and for chemical industry workers of the same age, we found that in the examined lymphocyte subpopulations only the total T-lymphocyte (T) population was by 10% lower ($p < 0.05$) in patients as compared to the same index in workers. At the same time, five or six indices were lowered at the first comparison with the donors' immunological indices.

Thus, under the effect of the damaging factors of chemical industry, by the suppression mechanisms the immune response of the organism was closer to the immune response of oncological patients. For example, in workers PhN was by 12% higher ($p < 0.05$) than in oncological patients. Chemical industry workers showed a tendency to a higher sensibilization of leukocytes to 100% endotoxin than did patients. Workers had higher blood serum total IgG and total IgM levels (by 23% and 45%, respectively, $p < 0.05$) as compared to oncological patients. Because of suppression, the specific IgMNA to endotoxin index in both groups showed no differences. However, PLNA% examination in the age groups showed an increasing tendency in workers aged 35–50 years. This response was suppressed in the 50–65 age group. Thus, tests of blood serum IgMNA to endotoxin revealed a higher ability of compensatory immune responses in workers of chemical industry than in oncological patients [40–42].

A comparison of data on oncological patients aged 50–85 years and on age-matched chronic patients (non-oncological patients were of this age group only) showed a lower (by 28%) lymphocyte count ($p < 0.05$). Because chronic non-oncological pathology exerted also a suppressive effect on immune response, T- and B-lymphocyte counts showed only a lowering tendency in oncological patients. Also immunological indices of compensatory reaction in this case were more pronounced than the same indices in oncological patients. For instance, PhN in chronic patients was statistically significantly higher (by 10%), while PhI did not differ, because it was compensated. In this comparison, neither PLNA% showed any differences. However, PLNA% in non-oncological patients aged 50–65 years was by 31% lower as compared to the same index in age-matched donors ($p < 0.05$) and did not differ from this index of onclogical patients and chemical industry workers. Thus, PLNA% was suppressed when leukocytes were sensitized to endotoxin. At 100% endotoxin, the MI was lower in non-oncological patients, i.e. leukocyte sensitization to this antigen was higher in these patients by 46% ($p < 0.05$). Compensatory response in oncological patients was manifested only by total IgG whose concentration in

blood serum was statistically reliably higher (by 15%) as compared to the same index in non-oncological patients ($p < 0.05$) [40–42].

DISCUSSION

Immunological and other specific reactions (natural and acquired) of the organism modify its sensitivity to cancer or other diseases depending upon environmental factors, carcinogens or other cell-damaging substances. The principle of their activity is to decrease the organism's predisposition to the disease or to increase its resistance to it. Though the majority of carcinogens are unable to cause risk effects immediately, the metabolism of these compounds is decisive in the host's response to the influence of the modified environment and in the adaptation of damaged cells to survive under unfavourable conditions [9, 10]. Many metabolic enzymes clearly demonstrate the genetic polymorphism of a population. The specific properties which express the increased sensitivity of the organism to diseases can be used to state the environmental contacts influencing the risk to catch one or another disease in sensitive individuals.

Earlier the synthesis of humoral antibodies has been supposed to be suppressed in response to the primary antigen stimulus in patients suffering from tumours of the reticuloendothelic system. It has been concluded that the application of primary antigens in such investigations is not expedient, because the organism with age acquires a wide immunologic reactivity connected with the action of many antigens: cross-reactions arise, which impede the evaluation of data [56].

The WHO recommends to determine serum immunoglobulins according to separate classes when suspecting primary and secondary insufficiency of the immune function. The level of serum immunoglobulins can change when influenced by age, work and living conditions, treatment and the progress of illness. We could add here that data found in the literature and proved by our investigations clearly demonstrate the importance of natural antibodies for evaluating the parameters of the humoral immunity chain [5, 40, 55, 56]. Their priority to immunal antibodies is supported by their natural presence in the blood serum of all mammalia (maybe their origin is evolutionary), whereas investigation of immunal antibodies requires immunization of the organism by a strange albumen. Artificial immunization limits the possibilities of repeated investigation in the experiment, especially in the clinic. For evaluating humoral immunity, the level of natural antibodies to the environmental antigens is more suitable [56].

Our many-year investigation data have proven that the level of specific IgMNA to endotoxin, both in the serum of rats and practically healthy humans, increases with age [23, 28, 36, 38, 39]. They also demonstrate the compensatory reactions of immune homeostasis occurring in the ageing organism or under the influence of damaging factors – carcinogenic and cytostatic substances [23, 55, 57]. It should be noted that this test is convenient for investigating the humoral immunity both in experimental animals and in practically healthy people and patients. The accuracy, sufficient sensitivity, comparative simplicity and the possibility to repeat the investigation many times with the same individual demonstrate the advantage of this method over the methods in which immune antibodies are used.

We found that oncological patients with decreased leukocyte and lymphocyte counts after chemotherapy and a decreased level of natural antibodies (specific IgMNA) to endotoxin in peripheral blood demonstrated much worse indices of their subjective and objective state than did patients who had a normal number of leukocytes and lymphocytes and an unchanged or increased specific IgMNA to endotoxin level ($\chi^2 = 5.7$; 5.5; $p < 0.05$) [5].

Endotoxin is isolated from gram-negative bacteria of the family Enterobacteriaceae naturally living in the digestive tract of man and most of other mammalia. In 1962, Kunin was the first to isolate from *Escherichia coli 014* and to describe the enterobacterial common antigen. He called it common antigen (CA) [58, 59]. The antigen was characterized in detail by Mäkelä and Mayer [60] who called it enterobacterial common antigen (ECA). They found that numerous Enterobacteriaceae strains give a cross-reaction with the antiserum to lypopolysaccharide (LPS) of *E. coli 04*. Antibodies to LPS without long-term immunization are ascribed to IgM [61]. Moncevičiūtė-Eringienė started investigations of IgM class NA to ECA (enterobacterial endotoxin) since 1970 by exposing experimental animals to carcinogenic and cytotoxic substances. Alongside the original regularities determined by herself, she confirmed also the above-mentioned literature data [3, 4, 13, 23].

Lugowski and co-workers also investigated ECA [22, 62]. After several years, Scott and Barclay [63] improved the conditions of enzyme-linked immunosorbent assay (ELISA) for IgG antibodies to gram-negative endotoxin glycolipids using LPS-polymyxin complexes. In 1995, Barclay et al. [64] called NA to ECA endogenous endotoxin-core antibodies (EndoCab). EndoCab ELISA is indicated as a practical test applicable for determining the presence of endotoxin [65]. The IgM EndoCab test can be also of prognostic value. If IgMNA synthesis is suppressed, the disease can have an unfavourable clinical outcome due to complications or higher mortality caused by endotoxemia. In this trend, numerous works have been published in relation to cardiosurgery, general surgery, sepsis, pancreatitis, etc. The test is regarded as an independent predictor of adverse postoperative outcome [65–68].

However, there are almost no works in which the EndoCab test is applied to prognosticate the outcome of oncological diseases after chemotherapeutical or surgical treatment. As we have stated in our work, the organism with an oncological disease is characterized by the presence of a deeper cellular immunosuppression. Besides, also its compensatory functions are more

suppressed than in cases of other chronic diseases or harmful professional exposure, although natural IgM class antibodies to endotoxin show the compensated protective function of the organism. Evaluation of the competence of this function as the self-protection of the organism from cellular damage using the other compensatory functions interconnected through endotoxin and a specific IgMNA to endotoxin should be regarded as more reliable and informative than when only one index (EndoCab) is used.

The application of a complex of compensatory immune reactions is of particular significance for natural endotoxin immunity state assessment in cases when chronic immunosuppression, oxidative, psychological and operative stress are present together. Without compensatory reactions the immune system is unable to manifest the whole totality of immunoregulatory reactions important for the organism's survival [40]. We suggest, in presence of oncological immunosuppression, to investigate more of the organism's natural immune functions related to specific IgMNA to endotoxin and to endotoxin itself, which is found in the internal surface of the external membrane of enterobacteria [28, 40, 41, 55].

We have found that in pharynx and hypopharynx cancer patients and in humans ill with chronic diseases or working in chemical industry, in cases when some of classic cellular type immunological functions are suppressed, the other both cellular and humoral immunological functions are stimulated. The degree of their manifestation was different and depended on pathological mechanisms. One of the authors (E.M.-E.) proposed a method for assessing stimulated-compensatory reactions, which included, alongside their complex (specific IgMNA to endotoxin, phagocytosis, LMIR and LAIR), a common substrate, enterobacterial endotoxin, and the component that reflects it, such as human blood antibodies to endotoxin and *Alcaligens faecalis 415*. The IgMNA to endotoxin test was proposed at our laboratory by E. Moncevičiūtė-Eringienė [11, 16]. The complex of reactions contained also the total IgM and total IgG indices. In our work, as far back as 1987 these reactions were found to be closely interdependent [17, 41, 55]. This interdependence is shown in Fig. 7.

The compensatory functions of the immune system are clearly related to age: as a rule, with age the specific IgMNA level in blood serum increases, whereas the classic cellular immunity functions become weaker with age [5, 36]. Additional investigations of the traditional indices of cellular and humoral immunity confirmed the validity of the proposed method of natural endotoxin immunity investigation [39, 69]. This complex of compensatory reactions is intended for determining the compensatory potential that supports the organism's immune homeostasis in the presence of immunosuppression [70].

The immunosuppression and immunostimulation states of the immune system in many cases are regarded as two separate phenomena that follow each other. Often immunostimulation follows immunosuppression when the

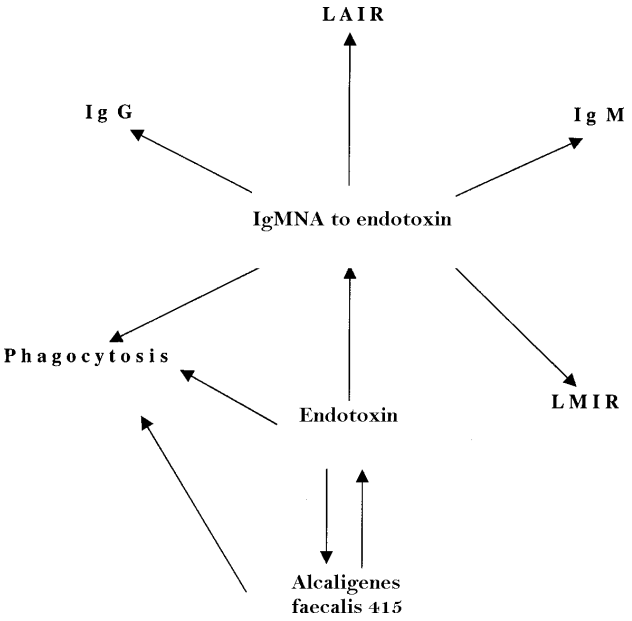

Fig. 7. Scheme of interaction of depressed immune state compensatory reactions with substrate of endotoxin and *Alcaligenes faecalis 415* (41, 55)

functioning of the immune system is compensatority restored, i.e. when its functions return to physiological norm [5, 23]. However, much more important is to explain spontaneous compensatory reactions taking place alongside suppressive reactions, even simultaneously. Such compensatory functions participate in maintaining the organism's homeostasis, – in this case immune homeostasis, when the organism is exposed to harmful factors or even when the pathological mechanisms are already in progress [11, 40–42, 69, 70]. In the immunoscreening methodology, the ANALL test-system can show a relationship of immune reactions and their compensatory possibilities in an aging organism or in that influenced by harmful factors [8].

CONCLUSIONS

The present review of our many-year investigations is intended to present the methodology of finding the natural antibodies to endotoxin from *Alcaligenes faecalis 415* and a conception of their integrated role in natural compensatory mechanisms of the immune system of the organism in cases when immune homeostasis is disturbed. Compensatory immune reactions of natural endotoxin immunity (LMIR, LAIR, phagocytic activity of neutrophils – phagocytosis, specific IgM NA to endotoxin, PLNA%, total IgM and total IgG) associated with the enterobacterial endotoxin and *Alcaligenes faecalis 415* are presented. A complex of compensatory reactions should be informative as to the prognosis of post-operative complications (higher mortality, prolonged hospitalization). The literature data on their application in oncological clinic are extremely scarce.

Interventions which increase the level of compensatory functions may improve the immune state of patients. In cases on suppressed compensatory immune functions they may help to foresee the risk of complications after chemotherapy or surgical treatment in oncological patients. In our studies, in patients with larynx and hypopharynx cancer before treatment, together with immunosuppression of cellular immunity these compensatory functions were also partly suppressed. In chronic patients with no oncological disease and in industrial workers exposed to chemical substances, compensatory functions were not changed or were most often stimulated, although the immunosuppression of these subjects was close to that observed in oncological patients.

It is possible to conjecture that the conception of natural immunity to endotoxin and the role of specific IgMNA to endotoxin in the compensatory mechanism of human immune state may be helpful in treating oncological patients. The possibility to apply this complex of reactions for prognosticating the postoperative complications in cancer surgery and other treatment situations is valuable in the prognosis of postoperative complications and mortality. Also, it is possible to prognosticate a relationship between the preoperative endotoxin immune status and the mechanism of spreading of cancer cells to other tissues in the perioperative period. The activated compensatory mechanisms of the natural endotoxin immunity may be helpful in improving the results of oncological patients' treatment and survival.

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References

1. Hirszfeld L. Über die Konstitutionsserologie im Zusammenhang mit der Blutgruppenforschung. Ergebn Hyg Bact Immun 1926; 8: 367–485.
2. Moncevičiūtė-Eringienė EV. On the resistance of cells to damaging factors. Pat Fiziol 1972; 6: 83–9 (in Russian, summary in English).
3. Moncevičiūtė-Eringienė EV. The appearance of antibodies to one of the common antigens of microorganisms of the intestinal group after administration to animals of carcinogenic agents and in anacidic gastritis in man. Pat Fiziol 1974; 5: 38–44 (in Russian, summary in English).
4. Moncevičiūtė-Eringienė EV. Changes in the synthesis of natural antibodies to one of the common enterobacterial antigens in carcino- and oncogenesis in rats and man. Vestn AMN SSSR 1978; 12: 69–71 (in Russian, summary in English).
5. Moncevičiūtė-Eringienė EV. Possible approaches to studies on immunological status in cancer patients and in healthy people. Exp Oncol 1984; 6: 8–14 (in Russian).
6. Moncevičiūtė-Eringienė EV. Actual problems of modern oncology in sanitation of the population. In: Moncevičiūtė-Eringienė EV, ed. Immunological Reactions of Organism and Their Possible Significance in Cancer Screening System. Vilnius: Mokslas. 1985; 6–21 (in Russian).
7. Moncevičiūtė-Eringienė EV. Novelties in the etiopathogenetic mechanisms of cancer. In: Moncevičiūtė-Eringienė E. V., ed. Immunological Imbalance Criteria as Possible Cancer Risk Factors. Vilnius: Mokslas. 1986; 7–23 (in Russian).
8. Moncevičiūtė-Eringienė E. The test-system ANALL and types of human immune state. Acta Med Lit 1999; 2: 147–56.
9. Moncevičiūtė-Eringienė E. Evolutionary malignant resistance of cells to damaging factors as a common biological defence mechanism in neoplastic development. Review of conception. J Exp Clin Cancer Res 2000; 19(3): 335–48.
10. Moncevičiūtė-Eringienė E. Neoplastic growth: The consequence of evolutionary malignant resistance to chronic damage for survival of cells (review of a new theory of the origin of cancer). Med Hypotheses 2005; 65(3): 595–604.
11. Kemeklienė R, Kazbarienė B, Milašienė V, Characiejus D, Moncevičiūtė-Eringienė E. Method for Definition of Compensatory Reactions of Human Immune System Functions (methodical recommendation). Lithuanian Oncology Centre, Vilnius, 1997, p. 19 (in Lithuanian).
12. Moncevičiūtė-Eringienė E. Activities of the Laboratories of Pathophysiology and Immunology over a period of 40 years. Medicina 1997; 10 (33): 41–54. (in Lithuanian, summary in English).
13. Moncevičiūtė-Eringienė EV. Two methods of obtaining specific antisera to common enterobacterial antigen. 1980; Lab Delo 2: 82–4. (in Russian, summary in English).
14. Moncevičiūtė-Eringienė EV, Bumelis VAB. Derivation and evaluation of purified common enterobacterial antigen necessary for determination of its natural antibodies in the blood serum of oncological patients. Exp Oncol 1991; 1: 41–5. (in Russian, summary in English).
15. Moncevičiūtė-Eringienė E. The change of microflora in association by antibiotic therapy of festering peritonitis. Vilnius: Dissertation. 1958 (in Lithuanian).
16. Moncevičiūtė-Eringienė E. The Method for Accelerated Evaluation of the Immune State of Man (methodical recommendation). Lithuanian Oncology Centre, Vilnius. 1986; p. 13 (in Russian).
17. Moncevičiūtė-Eringienė EV, Kazbarienė BA, Zaleskis GP, Milašienė VE, Characiejus DA. The application of the Common Enterobacterial Antigen for Elucidation of Different Variants of Immune Reactions (methodical recommendation). Lithuanian Oncology Centre, Vilnius. 1987; p. 10 (in Russian).
18. Ouchterlony O. *In vitro* method for testing the toxin producing capacity of diphtheria bacteria. Acta Path et Microb Scand 1948; 25: 186–191.
19. Gusev AU, Cvetkov BC. Micromethod of precipitation in gel-diffusion and its essential variants. In: Immunochemical Analysis, Moscow, 1968: 99–119 (in Russian).
20. Elgort DA, Abelev GI. Immunoautoradiographical definition of α-fetoprotein in animals and man. Biul eksperim biol 1971; 2: 118–20 (in Russian).
21. Kemeklienė R. ELISA studies for detection of natural antibodies to enterobacterial common antigen in human blood. In: International Scientific Conference "Laboratory and Clinical Immunology" (Abstracts). Vilnius–Birštonas. 1996; 31.

22. Lugowski Cz, Romanowska E. Characterization of an enterobacterial common antigen (ECA) epitope recognized by anti-ECA-tetanus toxoid conjugate serum. FEMS Microbiol Letters 1991; 77: 315–8.
23. Moncevičiūtė-Eringienė EV. ECA-test as an index of changes in immune system. In: Moncevičiūtė-Eringienė EV, ed. Immune Reactions of Organism and Their Possible Significance in Cancer Screening System. Vilnius: Mokslas. 1985: 29–48 (in Russian).
24. Spesivtseva EI. Phagocytosis on diphtheria. J MEJ 1952; 9: 12–6 (in Russian).
25. Nogachevskij II. Experimental Study of Immunological Reactions by Vaccination on Typhoid. Kiev: Autoref. of dissertation. 1961; p. 19 (in Russian).
26. Harington JT, Stastny P. Macrophage migration from an agarose droplet: development of a micromethod for assay of delayed hypersensitivity. J Immunol 1973; 110: 752–9.
27. Holan V. Antigen mediated macrophage adherence inhibition. Cell Immunol 1974;.13: 107–16.
28. Kazbarienė BA. Leukocytes adherence inhibition reaction and phagocytosis of neutrophiles in accelerated definition of immune reactivity in the system of cancer prevention. Vilnius: Dissertation, 1989 (in Russian).
29. Moncevičiūtė-Eringienė EV, Milašienė VE, Kazbarienė BA, Characiejus DA, Zaleskis GP. Normalization of suppressed immune state. In: Moncevičiūtė-Eringienė EV, ed. Immune Reactions of Organism and Their Possible Significance in Cancer Screening System. Vilnius: Mokslas. 1985; 63–124 (in Russian).
30. Mancini C, Carbonara A, Hermens J. Immunochemical quantitation of antigen by single radial diffusion. Immunochemistry 1965; 20: 235–48.
31. Stites DP. Clinical laboratory methods for detection of cellular immune function. In: Stites DP, Stobo JD, Fudenberg HH, Wells JV, eds. Basic and Clinical Immunology, V edition. Los Altos, California: Lange Medical Publications. 1984; 353–72.
32. Schütt H. Reaction of blast-transformation of lymphocytes (RBTL) in clinical diagnostics. In: Frimel H, ed. Immunological Methods. Moscow: Mir, 1979; 487–500 (in Russian).
33. Tessenov V. Reaction of blast-transformation of lymphocytes of the spleen by influence of antigen *in vivo*. In: Trimel H, ed. Immunological Methods. Moscow: Mir 1979; 159–64 (in Russian).
34. Johnson H, Lion F. The Statistics and Planning of Experiment in Technology and Science: Methods of Data Calculation (transl from English), Moscow, 1980 (in Russian).
35. Moncevičiūtė-Eringienė E. Enhancement of synthesis of antibodies to common enterobacterial antigen as immune response against cancer during evolution of induced tumors in rats. Symp. “Experimental Models in Cancer immunotherapy” (Abstracts), Bucharest. 1975; 24.
36. Moncevičiūtė-Eringienė EV, Kazbarienė BA. Distribution of NA to ECA and leukocytes of peripheral blood in practically healthy persons. In: Moncevičiūtė-Eringienė EV, ed. Immune Reactions of Organism and Their Possible Significance in Cancer Screening System. Vilnius: Mokslas. 1985: 49–101 (in Russian, summary in English).
37. Moncevičiūtė-Eringienė EV, Milašienė VE, Characiejus DA, Zaleskis GP. Evaluation of Human Immune State in Dependence on Harmful Industrial and Negative Habitual Factors (metodical recommendation), Vilnius. 1987; p. 12 (in Russian).
38. Moncevičiūtė-Eringienė EV, Kazbarienė BA, Characiejus DA. Immune state in dependence on age factor. In: Moncevičiūtė-Eringienė EV, ed. Immunologic Imbalance Criteria as Possible Cancer Risk Factors. Vilnius: Mokslas. 1986; 35–48 (in Russian, summary in English).
39. Moncevičiūtė-Eringienė EV, Milašienė VE, Characiejus DA, Taraškiavičius PM. Disturbances of human immune state as possible oncological risk factors. Exper Oncol 1989; 11: 60–3 (in Russian, summary in English).
40. Moncevičiūtė-Eringienė EV, Kazbarienė BA, Milašienė VE, Kemeklienė RR, Characiejus DA, Levit LD. Evaluation of compensatory reactions by means of common enterobacterial antigen in suppressed human immune response. Immunologia 1991; 6: 48–51 (in Russian; summary in English).
41. Moncevičiūtė-Eringienė E. Studies of immune state of oncological patients and healthy people and their methodological aspects. Medicina of Lithuania 1991; 2: 25–31 (in Lithuanian).
42. Moncevičiūtė-Eringienė E, Kemeklienė R, Milašienė V, Kazbarienė B, Characiejus D. Immunological investigations of onclogical risk and of cancer patients. Medicina 1991; 11–12(27): 10–20 (in Lithuanian; summary in English).
43. Moncevičiūtė-Eringienė E, Kemeklienė R, Kazbarienė B, Merkytė I. Compensatory mechanisms of human immunodeficiency state. Acta Med Lit 1995; 2: 23–9.
44. Moncevičiūtė-Eringienė E, Kemeklienė R, Kazbarienė B, Merkytė I. Human immunodeficiency reactions to environmental contamination. Part 1. Acta Med Lit 1995; 2: 30–6.
45. Moncevičiūtė-Eringienė E, Kazbarienė B, Kemeklienė R, Merkytė I. Human immunodeficiency reactions to environmental contamination. Part 2. Acta Med Lit 1995; 3: 3–11.
46. Kemeklienė R, Kazbarienė B, Bastienė D, Moncevičiūtė-Eringienė E. Human immunodeficiency reactions to environmental contamination. Part 3. Acta Med Lit 1996; 3: 55–64.
47. Kazbarienė B, Kemeklienė R, Bastienė D, et al. Human immunodeficiency reactions to environmental contamination. Part 4. Acta Med Lit 1997; 3: 3–12.
48. Kazbarienė B, Kemeklienė R, Šukytė Z, et al. Human immunodeficiency reactions to environmental contamination. Part 5. Acta Med Lit 1998; 3: 213–9.
49. Tamošiūnas V, Moncevičiūtė-Eringienė E, Čaplinskas S, et al. Alterations in the immune state of humans and animals and the spread of viral infections in districts contaminated in different ways. In: Kairiūkštis L, Rudzikas Z, eds. Ecological Sustainability of Lithuania (ECOSLIT). Vilnius: Lithuanian Academy of Sciences. 1997; 94–105 (in Lithuanian, summary in English).
50. Characiejus DA, Moncevičiūtė-Eringienė EV. Significance of serum immunoglobulins for support of immune homeo-

stasis. In: Moncevičiūtė-Eringienė EV, ed. Immune Reactions of the Organism and Their Possible significance in Cancer Screening System. Vilnius: Mokslas. 1985; 101–14 (in Russian; summary in English).
51. Žukauskas A, Jaskelevičius A, Stumbras A, Žiugžda J. Dynamics of pollutants exhaust in historical aspect and its geographical distribution. In: Kairiūkštis L, Rudzikas Z, eds. Ecological Sustainability of Regional Development in Historical Context (ECOSLIT). Vilnius: Lithuanian Academy of Sciences. 1996; 34–44 (in Lithuanian, summary in English).
52. Girgždys A, Šopauskienė D, Būdvytytė D, et al. Transformation of pollutants in the environment. In: Kairiūkštis L, Rudzikas Z, eds. Ecological Sustainability of Lithuania (ECOSLIT). Vilnius: Lithuanian Academy of Sciences. 1997; 17–29.
53. Moncevičiūtė-Eringienė EV, Milašienė VE, Characiejus DA, Kazbarienė BA, Zaleskis GP. Definition of Immunoniodulating Negative Action Under the Influence of Tobacco and Alcohol (methodical recommendation). Vilnius, 1987; p. 12 (in Lithuanian).
54. Moncevičiūtė-Eringienė EV, Milašienė VE, Kazbarienė BA, et al. Immunomodulating effects of tobacco smoking and alcohol consumption and oncological risk. In: Inductors of Interferon and Other Immunomodulators in Radiology and Oncology. Obninsk, 1989; 115–21 (in Russian).
55. Moncevičiūtė-Eringienė E. Smoking, alcohol, immunity and cancer. Vilnius: Mokslas. 1994; p. 255 (in Lithuanian, summaries in English and Russian).
56. Harris JE. The investigation of immune function in cancer patients. In: Scientific Foundation of Oncology. London: William Heinemann Medical Books LTD. 1976; 532–7.
57. Moncevičiūtė-Eringienė EV. Method of evaluation of the immune state of organism under the effect of immunosuppressors. In: Biochemical Institute of Lithuanian Academy of Sciences. The Evaluation of Cell Function. Vilnius. 1984; 175–88 (in Russian, summary in English).
58. Kunin CM, Beard MV, Halmagyi NE. Evidence for a common hapten associated with endotoxin fractions of *E. coli* and other Enterobacteriaceae. Proc Soc Exp Biol Med 1962; 111: 160–6.
59. Kunin CM. Separation, characterization and biological significance of a common antigen in Enterobacteriaceae. J Exp Med 1963; 118: 565–86.
60. Mäkela PH, Mayer H. Enterobacterial common antigen. Bact Reviews 1976; 10: 591–632.
61. Möller G, Sjöberg O, Anderson J. Immunogenicity, tolerogenicity and mitogenicity of lipopolysaccharides. J Infect Dis 1973; 128: 552–5.
62. Dell A, Oates J, Lugowski Cz, et al. The enterobacterial common antigen, a cyclic polysaccharide. Carbohydr Res 1984; 133: 95–104.
63. Scott BB, Barclay GR. Endotoxin-polymyxin complexes in an improved enzyme-linked immunosorbent assay for IgG antibodies in blood donor sera to gramm-negative endotoxin-core glycolipids. Vox Sang 1987; 52(4): 272–80.
64. Barclay GR. Antibodies to endotoxin in health and disease. Rev Med Microbiol 1990; 1: 133–44.
65. Barclay GR. Endogenous endotoxin-core antibody (EndoCab) as a marker of endotoxin exposure and a prognostic indicator: a review. Prog Clin Biol Res. 1995; 392: 263–72.
66. Bennett-Guerrero E, Ayuso L, Hamilton-Davies C et al. Relationship of preoperative antiendotoxin antibodies and adverse outcomes following cardiac surgery. JAMA 1997; 277(8): 646–50.
67. Bennett-Guerrero E, Panah MH, Barclay GR, et al.: Decreased endotoxin immunity is associated with greater mortality and / or prolonged hospitalization after surgery. Anesthesiology 2001; 94(6): 992–8.
68. Martinez J, Palazon JM, Munoz C, et al. Endotoxin and anti-endotoxin antibodies in the prognosis of acute pancreatitis. Rev Esp Enform Dig 2002; 94(7): 406–16.
69. Tamošiūnas V, Moncevičiūtė-Eringienė E, Kazbarienė B, et al. The early assessement of immunodeficiency in human and animals. In: Ecological Sustainability of Lithuania. Kairiūkštis L, Rudzikas Z, eds. Lithuanian Academy of Sciences, Vilnius. 1994: 49–56.
70. Tamošiūnas V, Moncevičiūtė-Eringienė E, Palaškienė Z, et al. Changes in the immune state of humans and animals and prevalence of viral infections after impact of pollution. In: Ecological Sustainability of Lithuania in a Historical Perspective. Kairiūkštis L, Rudzikas Z, eds. Lithuanian Academy of Sciences, Vilnius. 1999: 571–607 (in Lithuanian, summary in English).

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SUPRESUOTOS ORGANIZMO IMUNINĖS SISTEMOS KOMPENSACINĖS FUNKCIJOS EKSPERIMENTINĖJE IR KLINIKINĖJE ONKOLOGIJOJE: ENDOTOKSINUI NATŪRALIŲ ANTIKŪNŲ POVEIKIS (NAUJOS KONCEPCIJOS IR METODOLOGINIŲ ASPEKTŲ APŽVALGA)

Santrauka

Apžvalgos tikslas – pateikti naują koncepciją, kad endotoksinui specifiniai IgMNA atstovauja slopinamos imuninės sistemos natūraliems kompensaciniams mechanizmams. Daugelio metų (pradedant nuo 1970 m.) natūralaus imuniteto tyrimai, atlikti Vilniaus universiteto Onkologijos instituto Profilaktinės imunologijos laboratorijoje, rodo, kad tiek stimuliuotos, tiek ir supresuotos imuninės funkcijos yra glaudžiai susietos su IgM klasės natūraliais antikūnais (NA) prieš enterobakterinį bendrąjį antigeną (EBA), esantį žarninės grupės mikroorganizmų ląstelių išorinės membranos vidiniame sluoksnyje, t. y. su endotoksinui specifiniais endogeniniais antikūnais. Į tyrimą buvo įtraukta 1720 žiurkių, 4400 praktiškai sveikų žmonių, 60 dirbančiųjų chemijos pramonėje, 85 onkologiniai ligoniai ir 52 sergantieji kitomis lėtinėmis ligomis. Buvo panaudoti dviejų tipų imunologiniai metodai. Pirma metodų grupė (precipitacija agare, imunoautoradiografija, fagocitozė, leukocitų migracijos slopinimo ir leukocitų prilipimo slopinimo reakcija, bendrųjų IgM ir IgG koncentracijos nustatymas kraujo serume) buvo skirta nustatyti

endogenines kompensacines reakcijas endotoksino imunitetui, į kurių sudėtį įeina endotoksinui specifiniai IgMNA arba endotoksinas, arba pati bakterija Alcaligenes faecalis 415. Antros grupės metodais (netiesioginė imunofluorescencija ir blasttransformacija) buvo nustatyta ląstelinio imuniteto supresija. Apžvalga apima endotoksino ir kontrolinių serumų su šiam antigenui specifiniais IgMNA paruošimo metodikas. Aprašomi endotoksinui IgMNA specifiniai pokyčiai tirtų žiurkių kraujo serume priklausomai nuo kancerogeninių medžiagų ir chemoterapinių preparatų dozės. Analizuojama minėtų kompleksinių imuninių reakcijų priklausomybė nuo žmonių amžiaus, rūkymo, alkoholio vartojimo, gyvenimo pramonės užterštoje aplinkoje, darbo kenksmingomis sąlygomis, taip pat nuo sergančiųjų onkologinėmis bei kitomis lėtinėmis ligomis imunosupresijos. Taigi laboratorijos darbų duomenimis, endotoksinui specifiniai IgMNA gerai tinka natūralaus endotoksino imuniteto kompensacinėms funkcijoms nustatyti, esant klasikinio ląstelinio imuniteto supresijos elementams. Ateityje turi būti plačiau įvertinta natūralaus imuniteto endotoksinui kompensacinių mechanizmų reikšmė gerinant onkologinių pacientų gydymo ir išgyvenimo rezultatus.

Raktažodžiai: natūralus imunitetas endotoksinui, ląstelinis imunitetas, humoralinis imunitetas, endotoksinui specifiniai IgMNA, kompensacinės imuninės funkcijos, imunosupresija ir imunostimuliacija, imuninė homeostazė.