POINT OF VIEW

NATURAL ANTIBODIES TO ENDOTOXIN IN EXPERIMENTAL AND CLINICAL ONCOLOGY

E. Moncevičiūtė-Eringienė*, B. Kazbarienė, V. Milašienė, D. Characiejus, R. Kemeklienė
Institute of Oncology, Vilnius University, Vilnius, Lithuania

The studies on natural endotoxin immunity, carried out at Preventive Immunology Laboratory of Institute of Oncology of Vilnius University have shown that the natural immune functions of both immunostimulated and immunosuppressed organisms are closely related to IgM class natural antibodies (IgMNA) to the enterobacterial common antigen (ECA) from membranes of intestinal microorganisms, i.e. endogenous or spontaneous antibodies to endotoxin. With aging, when cellular immunity functions deteriorate, the level of these NA to endotoxin (NAE) in blood serum increases. The population level of NAE is enhanced by carcinogens and chemotherapeutic drugs in dependence from their doses and downregulated by tobacco smoking, alcohol consumption, exposure to environmental pollution, professional exposure to harmful agents, and chronic diseases. IgMNAE reflects enhancement of the natural immune functions of the organism on the background of the suppressed functions of classic cellular immunity and suits well to our new conception of elucidating the natural compensatory responses of immune homeostasis related to immune response to endotoxin.
Key Words: natural endotoxin immunity, humoral immunity, cellular immunity, endotoxin, compensatory immune functions, immunosuppression, immunostimulation.

Natural antibodies (NA) to enterobacterial common antigen (ECA) of *Enterobacteriacea* and other compensatory functions of immune homeostasis linked with this phenomenon counterbalance the suppressed functions of cellular immunity [1]. ECA is isolated from gram-negative bacteria of the family *Enterobacteriaceae*, naturally living in human digestive tract. In 1962, Kunin isolated common antigen (CA) from *Escherichia coli 014* [2]. This antigen (ECA) was further characterized in detail by Mäkelä and Mayer [3] who find 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 [4]. Since 1970, our group had started investigations of IgM class NA to ECA by exposing experimental animals to carcinogenic and cytotoxic substances [5–8]. The NAE test was proposed by E. Moncevičiūtė-Eringienė [1, 9]. The complex of reactions contained also the total IgM and total IgG indices, and these reactions were found to be closely interdependent [10, 11]. This complex of reactions is intended for determination of compensatory potential that supports the organisms immune homeostasis in the presence of immunosuppression [12].

Lugowski and co-workers also investigated ECA [13]. After several years, Scott and Barclay [14] improved the conditions of enzyme-linked immunosorbent assay (ELISA) for IgG antibodies to gram-negative endotoxin glycolipids using LPS-polymyxin complexes. In 1995, Barclay called NA to ECA endogenous endotoxin core antibodies (EndoCab). EndoCab ELISA is indicated as a practical test applicable for determining the presence of endotoxin [15, 16]. 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. The test is regarded as an independent predictor of adverse postoperative outcome [16–18].

However, there are no works in which the EndoCab test is applied on prognosis of oncological diseases after chemotherapy or surgery.

As we have stated, cancer is associated with cellular immunosuppression and suppressed compensatory functions that are more pronounced than in the cases of chronic diseases or harmful professional exposure. The objective of the present review is to analyze the data of the research work carried out by the Laboratory of Preventive Immunology on the compensatory functions of spontaneous endotoxin immunity upon immunosuppression caused by cancer, aging, exposure to environmental pollution and chemical substances, tobacco smoking, alcohol consumption, and to propose an integrated approach to investigation of natural endotoxin immunity functions.

Natural antibodies to endotoxin in experimental oncology. In 1970–74, we have shown that with aging, the IgMNAE of *Alcaligenes faecalis 415* in animal blood serum increases. Upon experimental carcinogenesis, NAE were found in blood sera of Wistar rats in 60–70% of cases *versus* 15–20% in control group ($p < 0.05$) [5, 8]. In the later study, it was demonstrated that administration of carcinogens *in vivo* resulted in increased levels of NA to endoxin of *E. coli* and *Alcaligenes faecalis 415* ($58.7 \pm 3.6\%$ in experimental groups *versus* $20.8 \pm 4.8\%$ in control group) [8].

Administration of carcinogens (benzo(a)pyrene, methylcholantrene, N-nitrozodiethyamine) or cytostatics (vinblastin, 5-fluoruracyl, sarcolysin, 6-mercaptopurin, cyclophosphamide, methotrexate, lophenalium) *in vivo* caused primary immunostimulation, then — immunosuppression and secondary immunostimulation, as it was shown by NAE test [8].

It has been shown that NA levels were significantly elevated in 2-month-old rats after intraperitoneal administration of extract of transplantable tumors (91% *versus* 9% in control group) [8].

Natural endotoxin immunity in healthy human population. In the first series (1979), IgMNA to endotoxin was investigated in the blood serum of 998 healthy donors (20–34 years, n = 403 (group 1); 35–49 years, n = 380 (group 2); 50–64 years, n = 189 (group 3); 65–79 years, n = 26 (group 4)) according to age, sex, harmful work conditions and place of residence [19, 20]. 67% of them had natural antibodies. Comparison of the PLNA% (population level of natural antibodies) demonstrated that in groups 2, 3 and 4 that index was correspondingly higher by 10%, 18%, and 40% ($p < 0.05$) than in group 1.

In males under 50 years, natural antibodies were by 14% more rare than in females of the same age. 40% of males worked under partially harmful conditions *vs*. 1.5% of females. The NA index of 60% of males working in unharmful conditions was the same as that in females. In group 3 the PLNA reached 78% both in males and females [19]. More than 90% of donors lived in towns. The PLNA% was equal in rural and urban females [19]. It should be noted that NAE was suppressed by harmful work conditions [21] pointing on the formation of the immunosuppressive mechanism in males professionally exposed to harmful factors.

Factors of environmental contamination. In the city of Vilnius, in 1984–87 the quantity of air pollutants has been stably increasing. During this period investigations were carried out twice with a 2-year interval. The PLNA index was lower (n = 189 in 1984–85; n = 204 in 1986–87) by 14% and the absolute number of leukocytes was also lower by 10% [11, 22].

Influence 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%. In subjects smoking 15–20 cigarettes per day, leukocyte count was higher by up to 17%, PLNA was lower by 24%, and blood serum total IgM levels lower by 31% as compared to analogous indices in non-smokers [22, 23]. Tobacco smoking and alcohol consumption break the balance of the NAE [22]. A particular emphasis should be put on PLNA% in tobacco smokers aged 20–34 years: their PLNA was suppressed by 21% [20, 23, 24].

Natural immune functions of endotoxin immunity in cancer patients. Immune competence to endotoxin was investigated in 85 patients with laryngeal and hypopharyngeal cancer cured in the Vilnius University Institute of Oncology clinic in comparison with three control groups: healthy donors (n = 57), chemical industry workers (n = 60) and patients with chronic non-oncological diseases (n = 52). The results showed that the functions of cellular immunity of oncological patients were suppressed in comparison with that of age-matched control groups ($p < 0.05$) [11, 25, 26]. PLNA% in oncological patients studied independently of age did not differ from control index, but its numerical value was lower. In the group of patients with pharyngeal and hypopharyngeal cancer aged 50–65 years, PLNA was statistically significantly suppressed (by 39%) compared to the same index in age-matched donors ($p < 0.05$) [11, 25, 26]. A comparison of PLNA indices for chemical industry workers and for healthy donors revealed no differences, but only a tendency to suppression in subjects aged 50–65 years [11, 25, 26].

There were no difference in PLNA% between the groups of oncological patients aged 50–85 and age-matched patients with chronic diseases, but in the last case for patients aged 50–65, PLNA was by 31% lower than that in age-matched healthy donors ($p < 0.05$) and did not differ from this index in onclogical patients and chemical industry workers [11, 25, 26].

Oncological patients with decreased leukocyte and lymphocyte counts after chemotherapy and a decreased level of NAE in peripheral blood demonstrated much worsier indices of their subjective and objective state than did the patients who had a normal number of leukocytes and lymphocytes and an unchanged or increased NAE level ($x^2 = 5.7; 5.5; p < 0.05$) [27].

So, our studies have demonstrated that 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 of the literature and the results of our investigations clearly demonstrated the importance of natural antibodies for evaluating the parameters of the humoral immunity chain [21, 23, 27] as well as the accuracy, sensitivity, simplicity of EndoCab test.

Interventions, which increase the level of compensatory functions, may improve the immune state of oncological patients, may help to prevent the risk of complications after chemotherapy or surgery.

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ЕСТЕСТВЕННЫЕ АНТИТЕЛА К ЭНДОТОКСИНУ В ЭКСПЕРИМЕНТАЛЬНОЙ И КЛИНИЧЕСКОЙ ОНКОЛОГИИ

Исследования естественного иммунитета к эндотоксину, которые проводили в Лаборатории превентивной иммунологии Института онкологии Вильнюсского университета, показали, что естественные иммунные функции при иммуностимуляции и иммуносупрессии связаны с IgM классом природных антител (IgMNA) к общему энтеробактериальному антигену (ECA) мембран кишечной микрофлоры, то есть с эндогенными или спонтанными антителами к эндотоксину. По мере старения организма на фоне снижения функций клеточного иммунитета уровень NA к эндотоксину (NAE) в сыворотке крови возрастает. Под воздействием канцерогенов и химиотерапевтических препаратов популяционный уровень NAE повышается, однако снижается при воздействии таких факторов, как курение, потребление алкоголя, наличие хронических заболеваний, загрязнение окружающей среды и вредные условия производства. Показатели IgMNAE (PLNA%) отражают усиление естественных иммунных функций организма на фоне подавленных функций классического клеточного иммунитета в соответствии с нашей концепцией о природном компенсаторном ответе иммунного гомеостаза, связанного с иммунной реакцией эндотоксина.
Ключевые слова: естественный иммунитет к эндотоксину, гуморальный иммунитет, клеточный иммунитет, эндотоксин, компенсаторные иммунные функции, иммуносупрессия, иммуностимуляция.