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Global Role of Low Molecular Weight Nucleic Acids in Biological Systems

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Abstract- Some considerations and reports are made regarding personal scientific developments carried out by author V.M. Zemskov in partnership with colleagues team and my close colleague prof. Zemskov A.M. for many years, specifically, 50 years. This is a problem, to which almost entire life has been devoted. It relates to a completely new global consistent pattern that we managed to stumble upon in those distant years, and that is implemented in any biological systems - whether it's a higher or a lower organism, a human being, or various microbial and cellular populations. Realized by low molecular weight RNA or oligonucleotides of RNA.

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Global Role of Low Molecular Weight Nucleic Acids in Biological Systems

Zemskov, V.M. [α], Neymann, V. [σ], Zemskov, A. M. [ρ] & Pronko, K. N. [ω]

Abstract- **Some considerations and reports are made regarding personal scientific developments carried out by author V.M. Zemskov in partnership with colleagues team and my close colleague prof. Zemskov A.M. for many years, specifically, 50 years. This is a problem, to which almost entire life has been devoted. It relates to a completely new global consistent pattern that we managed to stumble upon in those distant years, and that is implemented in any biological systems - whether it's a higher or a lower organism, a human being, or various microbial and cellular populations. Realized by low molecular weight RNA or oligonucleotides of RNA.**

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I. Introduction

Somewhere in late sixties (1967-1968) I and my colleagues drew attention to several works of Dr. Werner Braun where it was demonstrated that DNA fragments (oligonucleotides, not nucleosides) addition to various microbial populations led to substantial changes within such populations that included the intensification of microorganisms reproduction and selection of virulent cell clones, even if initially their concentration was 0.001 %.

After a short time, populations turned out to be almost 100% virulent, whereas Dr. Braun even managed to find out several mechanisms of this process - predominant virulent cells selection in a mixed population, the formation of factors which suppressed bacteria avirulent clones reproduction, microbes respiration intensification, increased kinase systems activity, etc. The same phenomenon arose if along with microbes, DNA specimens were introduced into the body, which was the source of their kinase systems activation, etc. This phenomenon also emerged if, along with microbes, DNA specimens were introduced into the body, which source was not of importance. Moreover, if DNA specimens were introduced into animals before infection, they developed a strong resistance to several microbes infection, and if drugs were introduced together with various antigens, the immune response was enhanced. The same idea of immuno-enhancing (adjuvant) action of DNA specimens was confirmed by Dr. Nakano, Johnson, Schmidtke, et al.

I became very interested in this issue and started research in related spheres, but along with the application of various high or low molecular weight RNA obtained from yeast fungi, different animal organs, transfer RNA, informational RNA, or high molecular weight RNA, decomposed by RNases (ribonucleases). I tried to maximize the purification of RNA specimens by different methods and obtained extremely pure specimens, which didn't contain protein, DNA, or polysaccharides impurities. These specimens' activity even enhanced.

a) Microbial populations alterations and infection worsening

Me and my colleagues have established (Zemskov, 1969; 1970a,b; 1972; 1974b; 1975a; 1977c; Zemskov VM and Zemskov AM,1992a; Zemskov et al., 1974a; 1977a,b; 1978a,b; 1985a; 2007) that various RNAs also have potent affection microbial populations (list of key publications related to this issue is outlined). They caused the acceleration of reproduction of the following microorganisms in vitro- St. aureus, albus, and No. 209, Shigella flexneri, boydii, and sonnei, Y. pestis (vaccine strain), leptospira, causative agents of tularemia, Francisella, E. coli, salmonella, anaerobic bacteria, conditionally pathogenic enterobacteriaceae, Candida albicans, perfect fungi. If the control leptospira grew to a maximum concentration on the nutrient medium without causing turbidity in it for about a week, with the addition of RNA, the medium became cloudy after a day, and the concentration of microorganisms was maximum. The same happened with slowly growing tularemia pathogens. When cultivating bacteria in the medium with RNA, the increase of their virulence and antigenicity was observed. Moreover, virulent microorganisms turned out to have a larger response to specimen, weakly virulent and conditionally pathogenic, appeared to be less responding. In contrast, to control cultures which quickly died and lost their virulent properties, more effective bacteria survival and typical properties preservation was noted in annual storage in the RNA-containing mediums. Same processes developed in animals' bodies if they were infected with

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staphylococci, Shigella, pathogenic Escherichia coli, salmonella, and other pathogens. There was a tremendous acceleration of the lethal infection clinical course, an increase in the number of microbes in organs, and produced toxins. If mice were intradermally infected with a specifically titrated small dose of toxicogenic staphylococcus (strain 0-15), which didn't cause skin lesions, in case if staphylococcus was administered with RNA - extensive skin necrotic lesions developed. Passaging of pathogenic E.coli and Shigella at the same time with RNA via mouse organism led to the sharp increase of microbe virulence compared with passaging without RNA. Passaging of three aforementioned Shigella strains in RNA-containing medium significantly increased the microorganisms sensitivity to antibiotics such as laevomycetin (chloramphenicol), tetracycline, penicillin, streptomycin.

Such a wide list of microorganisms exposed to RNA could not be accidental and demonstrated only the fact that this phenomenon is universal and wide spread, possibly plays a significant role in the development of infections in the body and even in some biological aspects.

b) Tachyphylaxis induction and immune response enhancement

Our further works (Zemskov,1975b; Zemskov AM and Zemskov VM, 1992b; 1995b; Zemskov VM and Zemskov AM, 1992a; Zemskov et al., 1977a; 1978b; 1985a; 2007; 1978c; 1979; 1981a; 1988; 1989; 1995a; 2019; Kochergina et al., 1986) allowed to find out that RNA specimens from various sources turned out to be interferonogenes that were clearly shown, and caused a state of increased resistance to different viruses — we showed this on the mouse influenza viruses APR8, western and eastern encephalomyelitis in horses, and tick-borne encephalitis which mice were infected with. Oral and intranasal specimen administration routes turned out to be effective. RNA specimens created animals' resistance to most diverse pathogenic and highly pathogenic microorganisms – I have revealed that at following microorganisms - E.coli, pathogenic Salmonella (Typhimurium, enteritidis, typhi abdominalis), Shigella, Staphylococcus, Proteus vulgaris, Kl. Pneumoniae, B. subtilis, cholera vibrio, actinobacillus mallei, and pseudocolor, Pseudomonas aeruginosa, then it was demonstrated by my followers and students using other infection models. It is important that increased resistance to infection by pathogenic microorganisms occurred already 4 hours after drug administration and persisted for 72 hours after a single injection. Repeated administration of RNA specimens was not accompanied by the emergence of drug tolerance; their effectiveness only increased. Using very low doses of RNA, but many times, it was possible to reduce the stimulator dose by 100 times while maintaining its effectiveness. The oral route of administration has proved quite effective.

i. *Detoxification of bacterial toxins, elimination of toxicity of hormones, cytostatics, antibiotics*

It was revealed that RNA specimens suppress microbes reproduction in tissues, neutralize bacterial toxins, activate antibacterial defense factors - both cell-mediated and humoral, increase body ability to detoxify toxins. Conducting specific studies (Zemskov VM and Zemskov AM, 1992a; Zemskov et al., 1978b; 1985a; 1984a; Bogdanova, 1980), we managed to prove that RNA led to bacterial exotoxins neutralization (gas gangrene pathogen toxin, staphylococcus hemolysin, pathogenic E. coli endotoxins) in case of specimen administration before and even after already happened organism intoxication. Significantly, RNA oral administration turned out to be most effective.RNA removed the toxicity of hormones (prednisone), cytostatics (cyclophosphamide), antibiotics (penicillins), antihistamines (diphenhydramine), bacterial polysaccharides, etc. It was possible to demonstrate using not only animals but in the clinical practice as well, which I will further speak about.RNA specimens increased sensitivity to various antibiotics, and therefore their administration with RNA allowed a sharp decrease in the dose of antibiotics with the same or even greater effect. By the way, in the treatment of people with severe lung diseases or some autoimmune diseases, it was possible to completely remove the toxic effect of drugs (hormones, cytostatics) and "transform," for example, hormone-dependent bronchial asthma into hormone-independent, i.e., completely refuse from hormones use.

ii. *Immune response enhancement*

RNA (Zemskov, 1975b; Zemskov AM and Zemskov VM, 1992b; Zemskov et al., 1977a; 1978b,c; 1981a; 1985a; 1988; 1989; 1995a; 2007; 2019; Kochergina et al., 1986)enhanced formation of the immune response to the soluble (typhoid Vi antigens, tetanus toxoid) and corpuscular (sheep erythrocytes, bacterial corpuscular vaccines) antigens - antibody formation increased, they appeared earlier and lasted longer, transplantation immunity increased when donor skin was transplanted to recipients, "delayed-type hypersensitivity" (sheep erythrocytes antigens, methylated bovine serum albumin), and the manifestation of "immediate hypersensitivity" in the model of anaphylactic shock decreased, therefore, RNA in the future was useful in various manifestations of allergies. The same immune response was achieved by using a combination of RNA with a 2-3-fold lower dose of the vaccine than without RNA, and significantly restored immunity after X-rays exposure of animals and increased cellular immunity in low-response (red blood cells) BALB\c inbred line mice to level of highly responsive animals of the CBA inbred line that cannot be done without RNA. Therefore, in this case, it was

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possible to carry out the phenotypic correction of a low immune genetically determined response. The mechanism of the adjuvant action of RNA consisted in the formation of a complex with antigens and their facilitated penetration into macrophages that process antigens because RNA is polyanionic and electrostatic charge substance; in enhancing the migration and cooperation of T- and B-lymphocytes and bone marrow stem cells, protein synthesis, activation of T-helpers and precursors of T- and B-cells, suppression of antigen-specific T-suppressors.

iii. *Metabolism intensification*

Strong activation of phagocytic cells was noted (Zemskov AM and VM, 1987; Zemskov VM and AM, 1992a; Zemskov et al., 1985a; 2007; 1981a,b; 1985b,c; Shcherbakova et al., 1981) macrophages and neutrophils – increase of their spontaneous migration, pathogenic microorganisms killing, pinocytosis and phagocytosis, oxygen metabolism, expression of Fcγ receptors and integrin adhesion molecules, spreading on the substrate and adhesion, activation of enzymes involved in oxidative metabolism and cell detoxification (glutathione peroxidase) and enzymes of oxidative phosphorylation, glycolysis, Krebs cycle, urea cycle, and amino acids catabolism, hexose monophosphate shunt, lysosomal hydrolases and phosphatases, NAD-dependent dehydrogenase, mitochondrial enzymes and enzymes of fatty acid metabolism, etc. Macrophages increased in size; polyribosomes, mitochondria, and lysosomes increased in number. RNA specimens caused a very rapid migration of neutrophilic phagocytes into the bloodstream from the depot of the body, the number of which could be increased in animals in as little as 90 minutes after oral administration.

c) Clinical efficiency

It is clear that having discovered such powerful "biodynamic effects" (the term was introduced by Werner Brown) of RNA, we tried to find its officinal drugs in the Russian Pharmacopoeia and apply them in the clinical practice. Such a drug was found - it turned out to be sodium nucleinate, which was a sodium salt of low molecular weight yeast (baker's yeast used in the baking industry) RNA and was used to combat agranulocytosis and leukopenia. At that time, nothing was known about the immunomodulatory properties of this drug.

We prepared new pharmacopoeial instruction for Pharmacology Russian Committee and have confirmed it. By now (Zemskov VM and Zemskov AM, 2014a; Zemskov et al., 1989; 1995a; 1982a,b; 1993; 1994a,b,c; 2000; 2013; 2014b; 2016a; Kanchurina et al., 1995;Mayorov et al., 1992;Provotorov et al., 1984; Revishvili et al., 2018b) we have conducted very extensive clinical studies of actually 30 various disease nosological entities with participation of more than 10 000 patients.Nosology is very wide - chronic and acute infectious bacterial and viral diseases (pneumonia, bronchitis, acute respiratory viral diseases, sexually transmitted infections, simple and genital herpes, cytomegalovirus infection and infection caused by Epstein-Barr viruses, hepatitis), autoimmune processes, various inflammations, immunodeficiencies, surgical complications, and mental illnesses, skin diseases, pyoderma and furunculosis, slow viral infections, delayed tissue regeneration, trophic ulcers, stomach, and intestinal ulcers, allergies (bronchial asthma, asthmatoid bronchitis), chronic fatigue syndrome, intestinal dysbiosis, cirrhosis of the liver and alcoholism, male impotence, diabetes. We apply methods of so-called alternative therapy that consist of simultaneous application of immunosuppressive drugs and RNA specimens that allow reducing doses of antibiotics, hormones, cytostatics, toxic medicinal drugs, and decrease or completely reduce toxic impact of all drugs above without general treatment efficiency deterioration. This approach has proven itself in the treatment of severe autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis, glomerulonephritis, bronchial asthma, multiple sclerosis). Of course, we have published a large number of reports, about 25 scientific monographs. This specimen is commonly used in Russia.

We quite succeeded in the prevention of acute respiratory viral diseases in military contingents, in hazardous industries - organic synthesis enterprises, “hot” shops, chemical plants, electrolysis production, child care facilities, etc. A huge advantage of RNA specimens is the practical absence of contraindications and side effects; the fact that they are natural components of our body and the foods we take daily, all without exception contain RNA; RNA specimens are also administered orally. These specimens are small RNA fragments that do not carry genetic information and, in this respect, are completely safe.

d) Non-genetic heuristic role of low molecular weight nucleic acids. New conception

It seems that nature “invented” a compound that is not by chance present in all cells and organs of living creatures, in soil, food, water and air, microbial communities - and performs an important regulatory and creative role in biological systems. It is the ubiquity of these substances, their extensive and universal properties that make us assume this most important key function (not counting the determining genetic information !!) that is not yet fully understood and known by us, but which most likely participates in maintaining homeostasis, evolutionary processes, development, aging, etc. The content of nucleic acids (Zemskov AM and Zemskov VM, 1995b; Zemskov VM and Zemskov AM, 1992a; Zemskov et al., 1985a; 1995a) in food products is quite high, especially in animal products - in fish - 1.6%, beef liver - 24 %, pig kidney - 2.7%, etc. A

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person with a balanced diet receives about 1 g of nucleic acids per day. Nucleic acids in the soil are in a free state, unlike the cells - this means that the information fund of the biosphere is not inactive but performs an important function. There is an opinion that there is a complete exchange of information between all living things without their taxonomic restrictions (Zemskov et al., 1995a). It is very important that RNA molecules "combine" genetic, protein-synthetic, and enzymatic functions, and this is the deepest meaning of their participation in the exchange of information, processes of evolution, differentiation, and reproduction of cells, and other key processes.

It would seem that in microbial populations and the animal organism the effects of RNA are "opposite" - however, this assumption is erroneous and confirms only one thing - the mechanism of action of RNA is universal, and uniform at all levels — microbial populations change because their reproduction period is on average about 20 minutes, and somatic cells of the body - 24 hours. That is why, if microbes enter the body along with RNA, they multiply rapidly, causing infection, while somatic cells do not have time to strengthen their antimicrobial and immune power that requires a genetically programmed time. If the drug is administered before infection in a few hours, the cells manage to migrate, multiply, increase their functional activity, and then the microbe enters the prepared body and is not able to break through the immune defense.

Years of experience in this direction led me and my employees to a certain universal concept (Zemskov, 1970b; ZemskovAM and Zemskov VM, 1992b; 2016b; Zemskov VM and Zemskov AM, 2014a; Zemskov et al., 1984a; 1985a; 2007; 2019; 1994b; 2014b; Revishvili et al., 2018a,b), built based on non-genetic and non-informational properties of RNA and DNA that, it seems to me, may lead to completely unexpected and fundamental knowledge in the field of biology and medicine and new ideas about the development of infections, immune response, tissue homeostasis, etc. This approach may be marked by the development of completely new methods of treating infectious diseases that are not associated with either an effect on microorganisms or the body's immune system. It can also lead to the creation of fundamentally new therapeutic approaches that may be based on directed transport of activated body cells to foci of infection, pathology, or cancer targets. In this regard, I have already carried out preliminary experiments that confirm the correctness of the idea. Although, of course, there is still much work to do.

Unfortunately, due to objective circumstances, I have not yet managed to complete these final works.

However, there is no doubt that this problem will still arise, and it will be resolved in the future.

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