MICROBIOME AS A POSSIBLE CAUSE OF XXI CENTURY DISEASE

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Abstract - In the 21st century, diseases such as depression, obesity, autism, allergies, diabetes, autoimmune diseases are the most common affecting society, and in large part they are of uncertain etiology and lack efficient medical treatments. Therefore modern, medicine seeks to explain the reasons for their occurrence and to establish risk factors. Interesting observations are drawn from studying the impact of the human metagenom on the mechanisms occurring in the human body.

Keywords - Diseases of XXI Century, Microbiome, Human Metagenom

I. INTRODUCTION

The term “microbiome”; was proposed in 2001 by Joshua Lederberg to describe a set of all human microorganisms, both commensal, symbiotic and pathogenic, which inhabit the interior of the human body and occur on its surface. Currently, “microbiota”; means all microorganisms, i.e. fungi, bacteria, viruses and archaea, inhabiting the human body, while “microbiome”; means a collection of their genomes. The microbiome in the human body is located in specific places, e.g. skin, mouth and upper respiratory tract, reproductive system and digestive system. It is estimated that the number of cells in the microbiome exceeds 10-fold the number of cells building a healthy human body. The results of many studies have shown that microorganisms have the ability to communicate not only with each other, but also can conduct molecular dialogue with host cells.
Such a rich and multidirectional network of connections, provides the possibility of signal transmission and communication of microorganisms with each other, but also with host cells. Microorganisms together with host cells form a comprehensive interactive ecosystem that decides on many different biological processes, including health or disease [1].

II. IMPACT OF THE MICROBIOME ON 21ST CENTURY DISEASES

A. *Depression*

According to information provided by the World Health Organization by 2020, this disease may become the second most common disease in the world. It is also the most common mental disorder that affects 11-15% of the human population during their lifetime. This percentage increases when taking into account patients suffering from chronic diseases, which can also be associated with poorer property status, social isolation and disability. However, social factors do not fully explain the reason for which the frequency of depressive disorders is higher in relation to diseases with increased activity of the immune system. It is known that reactions to feelings of anxiety affect our internal organs and vice versa, the health condition of the latter can also affect anxiety and depression [2].
An experiment was conducted to investigate the potential antidepressant effect of *Bifidobacterium infantis* in rats, subjecting them to the Forced Swim Test (FST) [3]. The probiotic group had reduced levels of proinflammatory cytokines IFN-γ, TNF-α and IL-6, and decreased levels of IL-10 compared to the control group that was not fed probiotics in the diet. What's more, the probiotic had an effect on increasing the content of tryptophan and its metabolite - kynurenine acid, which has a neuroprotective effect through its antagonism to N-methyl-D-aspartic (NMDA) receptors. Another finding in this experiment was that in the group receiving the probiotic there was a reduced content of 5-HIAA (5-hydroxyindole acetic acid) – a product of serotonin transformation - in the frontal cortex, and a reduced content of DOPAC (3,4- 24 dihydroxyphenylacetic acid) - a dopamine metabolite – in the cortical part of the amygdala. Another study aimed to assess the potential antidepressant effect of *Bifidobacterium infantis* [5]. For this purpose, young rats were subjected to stress by separation from their mothers. The probiotic caused a clear normalization of the immune response, although to a slightly lesser extent than citalopram - an antidepressant.
The probiotic lowered IL-6, and reversed behavioral deficits and restored the initial concentration of noradrenaline in the brainstem.
Rodent studies have shown that in combination, *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 had anxiolytic activity, significantly reducing anxiety-related behavior in rats and alleviated the level of psychological stress in volunteers, including severity coefficients for somatization, anxiety, depression and aggression-hostility rate. The cortisol content also decreased [6].

B. *Obesity*

Intestinal microflora plays an important role in our nutrition. Bacteria are responsible for the synthesis of group B vitamins, vitamin K and for the breakdown of many complex molecules found in foods, such as meat and vegetables. They produce energy from plant food, which we consume, but also digest plant fibers into smaller particles that our body cannot digest on its own. Currently, there are more obese people in the world than malnourished, of whom the latter are increasing due to the escalating food crisis. According to the Red Cross in the world in 2010 there were 1.5 billion obese people and 925 million malnourished. Obesity has become an epidemic in highly developed countries and is a growing problem in developing countries. It is influenced by many factors, such as: genetic predisposition, low physical activity, too caloric food. It has become a serious problem due to the health consequences it causes. Among other things, it is one of the causes of type 2 diabetes, causes cardiovascular disease, sleep apnea, gastroesophageal reflux disease and cancer. These are just some of the disorders that result from being overweight. Obesity causes a disturbance of energy metabolism homeostasis, which is the result of an increase in body fat mass above normal. This is a consequence of excessive filling of existing fat cells (hypertrophy) or the formation of new fat cells (hyperplasia) with triacylglycerols, or is the effect of both at the same time. For the proper functioning of the human body, energy obtained in the metabolism of nutrients contained in food products is needed. The composition and weight of the body depend on the balance between the amount of energy supplied with food and its consumption. The energy balance and nutritional ratio, which is an expression of the ratio of carbohydrates to fats in food, is becoming increasingly important. The human demand for energy results from expenditure related to the basic transformation, which is necessary to maintain the body's physiological functions, thermogenesis and expenditure on muscle work. The consequence of the uncontrolled positive energy balance is the steady increase in the incidence of obesity among various human populations around the world. The main reason is increased food consumption and / or reduced energy expenditure. The impact of microflora on the development of obesity is currently the subject of many studies aimed at confirming the effect of intestinal microbes on weight gain, and understanding the mechanisms of their action can help prevent and treat people suffering from this disease. It is very likely that obesity can be managed by manipulating the composition of the bacterial flora in the gut [7,8,9].

In 2004, Backhed started working with a microbiome expert, Professor Jeffrey Gordon from Washington University in Saint Louis, Missouri [10]. They noticed that germ-free mice were disproportionately skinny. They argued that this could cause a lack of intestinal bacteria. They decided to conduct an experiment. They sprinkled the hair of these mice with the contents of the intestines taken from mice born by natural birth. Thanks to this procedure the bacteria were transferred to the intestines of germ-free mice. After this procedure, the mice increased their body weight by 60% within 14 days. However, the results of an experiment in which bacteria were taken from obese mice and transferred to the intestines of germ-free mice are evidence that the type of bacteria inhabiting the gastrointestinal tract varies depending on body weight. The second group of germ-free mice were given bacteria from rodents whose body weight was within the so-called "normal body weight". Both groups of germ-free rodents were then fed the same amount of the same food. After two weeks, the animals that received the "obese" microflora had much more fat tissue than mice that received the "lean" bacteria. This is clear evidence that the first group of mice's intestinal bacteria metabolized the food much more efficiently and produced metabolites that provide more calories to the mice's body than the second group of mice's intestinal bacteria. The intestines of obese people are dominated by bacteria belonging to the *Firmicutes* type, with a low G+C content, including more than 250 types of bacteria, including *L actobacillus*, *Enterococcus* and *Peptostreptococcus* , while the number of *Bacteroides* is decreasing. The human intestinal microflora decomposing fiber to short-chain fatty acids provides the body with 80 to 200 kcal per day. *F irmicutes* have the ability to use energy from various complex organic substances more efficiently and cause, among other things, increased fermentation of undigestible polysaccharides into absorbable forms. They also contribute to increased absorption of monosaccharides and short-chain fatty acids from the gastrointestinal tract, which (and sugars and acids) are then converted in the liver to fats and deposited in the fatty tissue. [13]

C. *Autism*

Autism is a disease of unexplained etiology, which was first described by Kanner in 1943. Characteristic for this disease is the spread and management of behavioural disorders, which usually manifest themselves in early childhood and persist in adulthood. Apart from neurological symptoms, people affected by this disease often suffer from gastrointestinal disorders, which suggests the influence of intestinal microflora on the pathophysiology of the gastrointestinal tract. Epidemiological data show that this disease occurs in 10-20 out of 10,000 children with a 4-fold prevalence of boys among those suffering from it. In some cases, chromosomal anomalies or metabolic disorders are described. Since the intestinal microflora plays a key role in the metabolism and maintenance of immune homeostasis and can control the activity of the Central Nervous System(CNS) through nervous,

hormonal and immunological pathways, a hypothesis of the active role of the intestinal microflora in the pathophysiology of autism spectrum disorders (ASD) has been put forward. The dominant bacteria in the intestinal microflora of autistic children are anaerobic bacteria, including *Clostridium* spp. Some canes are capable of producing toxins such as neurotoxins or enterotoxins [14, 15]. The hypothesis about the role of bacteria in the development of autism was first raised by Ellene Bolte in 1998. It was developed by a group of researchers led by Sydney Finegold from UCLA School of Medicine, USA thanks to a cycle of studies in which they assumed that some of the symptoms of autism may be related to the production of toxins or other metabolites by bacteria belonging to the species *Clostridium* spp. Residing in the digestive tract of children with autism. The observation of children by their parents has helped to make this hypothesis [16]. They observed in their children that after treatment with broad-spectrum antibiotics diarrhea was present, followed by speech loss and increasing tendency to isolation from the environment and development of further symptoms of autism. The above-mentioned observations led American scientists to conclude that frequent antibiotic therapy in these children may be an initiator of bacterial flora disorders leading to the proliferation of pathogenic toxin-producing bacteria. A study comparing the gastrointestinal microflora of healthy and autistic children showed significant differences between them. The study and control group also showed differences between the composition of the lower and upper gastrointestinal microflora. Samples of materials from the stomach and duodenum taken from healthy children did not contain microaerophilic and non-corulating anaerobic bacteria. The initial assumption of the authors about the search for toxins produced by the bacteria that act on the central nervous system nervus vagus, which is responsible for small intestine innervation, prompted the researchers to focus on the analysis of the composition of the upper gastrointestinal microflora in both groups - control and experimental/research. From faecal samples of the examined group of autistic children 5 species of bacteria belonging to the genus *Ruminococcus* and 23 different species of bacteria belonging to the genus *Clostridium* were cultured. The control group consisted of 5 and 15 types of bacteria respectively. The number of strains per sample in the experimental/test group was 2-10 and in the control group 3-12. In autistic children the total number of bacteria was 10**2** -109 and in healthy children 104 -108 . This is ten times higher average number of microorganisms in sick children. From the faecal sample of autistic children strains of nine species of the genus *Clostridium* were isolated, but not from the control group. Gram-positive rods, which produced subtemporal disputes, were among them. Genetic and biochemical tests of these previously unknown *Clostridium* species have shown that they have different properties from known strains of *Clostridium* species. The final products of glucose degradation by these bacteria are lactic acid and acetic acid. These bacteria do not have the capacity to hydrolysise esculin, urea and gelatine. Instead, they produce enzymes such as acid phosphatase, α-galactosidase.

In another study, bacterial and fungal intestinal microflora was characterized in autistic individuals, which showed changes in the composition of the intestinal microflora of autistic individuals compared to the intestinal microflora of healthy individuals. A significant increase in the ratio of *Firmicutes* to *Bacteroidetes* type bacteria was found in autistic subjects.

A decrease in the relative number of bacteria from the genera *Alistipes* , *Bilophila* , *Dialister* , *Parabacteroides* and *Veillonella* and the growth of bacteria from the genera *Collinsella* , *Corynebacterium* , *Dorea* , and *L actobacillus* were observed [17].

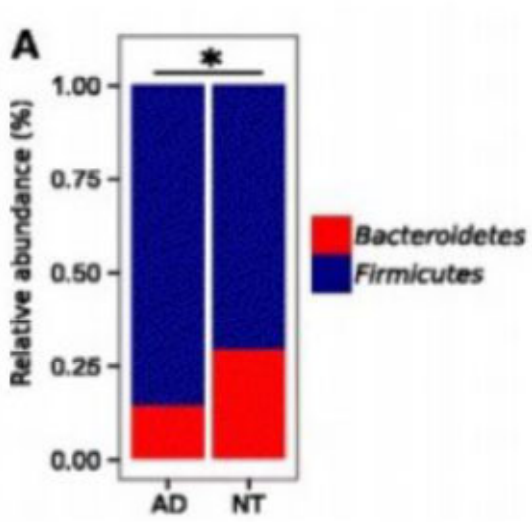


Fig. 1. Average relative amount (%) of *F irmicutes* and *Bacteroidetes* in autistic (AD) and neurotypical (NT) people; * p <0,005, Wilcoxon's test; the bacterial ratio (*Firmicutes/Bacteroidetes*) is on the indicator [15].

D. *Autoimmune disease*

Microorganisms residing in the human intestines form a link with the immune system. Specifically, they inhabit the epithelium lining the digestive system. They are in constant contact with the part of the immune system located in the intestines. The intestinal microflora also influences the systemic immune system, i.e. elements of the immune system circulating in the whole human body. The experiment carried out on gnobiotic mice bred in sterile conditions showed that intestinal microflora influences physiological processes in the intestines [18]. Germ-free rodents did not develop intestinal villi properly and enterocytes took on a cubic shape instead of a cylinder. Large amounts of mucus were secreted into the intestinal lumen due to the lack of

intestinal microflora regulating this process. Then the mice inoculated with the intestinal bacterial flora were observed to gradually rebuild their normal intestinal anatomy. Apart from these abnormalities, sterile mice had many immune system related defects. Weak development of Peyer's tufts, different composition of CD4+ T cells and B cells in the proper intestinal tray were found. From previous hypotheses on how the immune system differentiates its own cells from genetically different ones, it would appear that cellular defense mechanisms should constantly fight bacteria living in the human body. On the other hand, the human immune system recognizes microorganisms thanks to the group of PRR (Pattern Recognition Receptors) of congenital responses, which recognize molecular pathogenicity patterns of PAMP (Patogen Assocaited Molecular Patern). The natural intestinal flora being in the body all the time must reduce contact with the epithelium of the intestine and the communicating immune system to avoid the development of unnecessary inflammatory response. The response should be induced only by pathogens. The intestinal immune system GALT (Gut Associated Lymphoid Tissue), has produced only partially known mechanisms involved in maintaining the induction of an inflammatory response by intestinal commensals and antigens derived from food [19].

Commensal bacteria in reaction with the immune system show common features with pathogens. Components of their cell wall, such as LPS, i.e. lipopolysaccharide, peptidoglycan or lipotoxic acids, are recognized by membrane proteins, which are receptors of host cells, such as Toll-Like Receptor-TLR [21]. They are located on the surfaces of respiratory tract cells, intestinal epithelium, mast cells, dendritic cells, adipocytes and macrophages. During normal host response, a cycle of transformations is initiated by connecting them to corresponding molecular patterns. As a result of this reaction, the transcription factor NF-kB and cytokine promoter genes are activated. This results in mass production of cytokines and chemokines, synthesis of antibacterial peptides and activation of dendritic cells, which results in elimination of the microorganism. Similarly, endotoxin-activated macrophages (via TLR4) initiate the production of proinflammatory cytokines such as IL-12, IL-6, lL-8, iLL-1, TNF-α in just a few minutes. The production of defensins by enterocytes is a consequence of TLR activation contributing to NF-kB transactions [20]. A mouse study has shown that commensals induce a host-protective anti-inflammatory response using TLR. However, an inflammatory response that is harmful to the host is induced by these bacteria in the absence of the host's ability to produce IL-10 anti-inflammatory cytokine. It has also been observed that germ-free mice, which grew up in sterile conditions and had no microflora, had an abnormal immune system with weaker acting T-cell regulators. After inoculation of rodents with these bacteria, the correct balance between proinflammatory and anti-inflammatory lymphocytes was established and the immune system started to function normally/maintain properly. This is an example showing the balanced relationship between intestinal bacteria and the human body resulting from controlled stimulation of the immune system.

As a result, it is possible to develop a pro-inflammatory reaction when the bacteria or their products, for example, will penetrate beyond the intestinal boundaries or an anti-inflammatory host reaction when the microorganisms are within the intestines. An important direction of the study was the analysis of sugar molecules bound by glycosidic bonds to the surface of *Bacteroides fragilis* cells, thanks to which the immune system is able to recognize the presence of these bacteria. One of these molecules is polysaccharide A, which promotes the maturation of the immune system and also provides a signal for this system to increase the synthesis of regulatory T lymphocytes, which in turn inhibit the antibacterial activity of proinflammatory T lymphocytes.

Bacterial cells belonging to strains deprived of polysaccharide A do not survive in the intestine because the cells of the immune system attack them as if they were cells of pathogenic bacteria. The quantitative and qualitative disorders of human intestinal microflora may cause a higher incidence of many autoimmune diseases. The reason may be the lack of polysaccharide A (a compound that stimulates the immune system to produce more regulatory T lymphocytes), so natural killers (NKTs) of T lymphocytes begin to attack everything they encounter in their path, including their own tissues. The rapid, 7-8-fold increase in autoimmune diseases such as Crohn's disease, type 1 diabetes mellitus and multiple sclerosis is associated with a decrease in the number of beneficial microorganisms [22]. These diseases are genetically as well as environmentally determined, with the environmental factor being linked to microorganisms. Changes in the composition of the microorganisms have an impact on the immune system. Another example of interactions of bacteria providing intestinal homeostasis is the activity of individual *E nterococcus* granule strains that regulate phosphorylation of receptors activated by Peroxisome Proliferators-Activates Receptor gamma-PPAR. This pathway causes the induction of expression of target genes, including the genes responsible for the production of cytokine IL-10, which has an anti-inflammatory effect. The experiment, which deprived mice of PPAR-γ receptors, showed that rodents were unable to maintain intestinal homeostasis. At the current stages of the study, the correlations observed in humans between a reduction in the number of microbial infections and an increase in the incidence of immunological disorders remain only coincidental.

It is difficult to recognize what is the cause and what is the effect. Whether it is the loss of microorganisms that leads to a higher incidence of autoimmune diseases or diseases resulting from auto-aggression create unfavorable conditions for the development of microorganisms. It is more likely that the first statement that changes in the bacterial intestinal flora have an impact on the increase in the incidence of autoimmune diseases in humans [23, 24]. An example of common autoimmune diseases is bowel disease. Inflammatory Bowel Diseases (IBD) is a group of incurable and chronic gastrointestinal diseases characterized by specific remissions and relapses with unexplained etiology [25]. They belong to the group of auto-aggressive diseases, i.e. those resulting from the improper functioning of the immune system. These include ulcerative colitis, Leśnikowski-Crohn's disease and less frequent collagen colitis, Behcet's disease, acid-absorbent enteritis and ischemic colitis. It is suspected that the initiating factor in the development of inflammatory bowel disease may be the abnormal composition of the bacterial flora, such as a decrease in the number of *Bifidobacterium* and *L actobacillus*. This may be associated with the anti-inflammatory and regenerative effects of these bacteria on the intestinal mucosa and thus decrease its antigen permeability. IBD often starts after food poisoning or after antibiotic treatment. However, the most important cause of Leśnikowski-Crohn's disease and ulcerative intestinal inflammation is an imbalance of bacterial microflora leading to disorders of the immune system and intestinal inflammation. Intestinal epithelial cells – dendritic and Paneth's cells are sources of numerous antibacterial endogenous substances such as phospholipase, lysozymes and antimicrobial peptides: Regllly, angiogenin 4 or α-defensin. The Regllly factor is a type C lectin, which is expressed by a signal that is the result of TLR activation. *Bifidobacterium* rods are capable of inhibiting this signal.

On the other hand, NOD (Nucleotide-Binding Oligomerization Domain) proteins - receptors on host cells are capable of transmitting a signal that activates NF-kB protein independently of TLR receptors and are able to bind to polysaccharide and peptidoglycan. Peptidoglycan activates the NOD2 protein presented on Paneth cells and thus leads to α-defensin expression. Some symptoms of Leśnikowski-Crohn's disease can be correlated with a mutation of the gene encoding NOD2 protein. In the small intestines of Leśnikowski-Crohn's disease patients the expression of α-defensin is significantly inhibited.

However, such situation was not observed in patients with the disease involving the colon. Whereas *Bacteroides thetaiotaomicron* with PPAR-γ receptors cause redistribution of ReIA protein, which is a subunit of the NF-κB protein complex in the cytoplasm, which selectively weakens the inflammatory response. On the other hand, *Saccharomyces boulardii* producing a heat-stable, small factor, prevents degradation of IκBα protein, which limits the binding of NF-κB protein to DNA and weakens the expression of the gene encoding chemokine IL-8.

Bacteroides t hetaiotaomicron has a similar anti-inflammatory effect. This microbial species additionally induces the host to produce fucosylated glycans instead of sialylated glycans, as it uses fucose as its energy source. This is probably due to the Control of Signal Production (CSP), which activates the transcription of the fucosyltransferase genes. The above data show the ability of autochthonous bacteria and their metabolites to regulate the human anti-inflammatory response by interacting with the nuclear factor PPAR-γ.

The comparison between the microflora of healthy people and those suffering from IBD shows big differences.

In IBD patients, the study revealed differences in microbiome composition depending on the type of disease.

Patients with increased flatulence and rapid feeling of satiety had a higher number of microorganisms of the genus *Cyanobacteria* , and in those who felt more pain, more *Proteobacteria* cells were detected. Positive effects in patients were brought about by equalization of physiological composition of intestinal microflora. This has been confirmed in studies showing that applying the preparation containing specific strains of *L actobacillus* and *Bifidobacterium* and *S treptocococcus t hermophilius* bacteria, effective protection against recurrence of diverticulitis was achieved in patients with ulcerative colitis. In patients receiving the preparation with bacteria, the disease recurred only in 15%, and in the group taking placebo in 100% of the patients [26].

There is also a speculation that one of the possible causes of induction of an allergy is human microbiom's modification. Sjorgen et al. have shown that reducing the biodiversity of children's microbiome in the first two months of life is associated with greater the percentage of allergy incidences in the first five years of children's life.

Allergy is a sign of immune defense aggressiveness. The immune system reacts to antigens that the system should normally tolerate. Normally, substances administered orally are unnoticed and ignored by the immune system of the oral mucosa. This is an oral tolerance. Mechanisms involved in the development of oral tolerance are associated with the induction of lymphocyte immune response regulators [27].

Immunity cells activated by antigens secrete inhibitory cytokines, in particular IL-10 and TGF-γ. The presence of a healthy human microflora is required to induce an oral tolerance reaction. Germ-free mice did not show oral tolerance to antigens. The lack of lipopolysaccharides on the cell wall of Gram-negative bacteria is responsible for this phenomenon. In the period before the development of the industrial

revolution and excessive hygiene care, healthy newborns in the first days of their lives were colonized by germs from the *Enterobacteriaceae* family, mainly by *E scherichia c oli*. Due to the delayed colonization of Gram-negative bacteria in the digestive tract of children, the development of oral tolerance reactions is also delayed. The bacterial microflora of allergy sufferers from healthy children differs mainly in the number of lactic acid rods. These bacteria also have a therapeutic effect in allergic pimples. The crucial thing in this condition is the ability of bacteria to different induction of cytokine profile of type Th1 (IL-2, IFN-y) and type Th2 (IL-4, IL-5, IL-10, IL-13), as well as the ability to influence their balance. This is because bacteria selectively stimulate the Th1 response thanks to immunostimulating sequences contained in their DNA. Excessive hygiene, preventing normal colonization of the gastrointestinal tract by bacteria, leads to inhibition of the correct type Th1 immune response. Changes in the bacterial microbiome may also lead to the consolidation of preferences of the Th2 type immune response [28, 29].

III. CONCLUSION

Research on the microbiome of humans will undoubtedly contribute to a better understanding of the importance of indigenous microorganisms in the biology of higher organisms, including understanding of those relationships that contribute to maintaining health or developing the disease.

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