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Review

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The Effect of SARS-CoV-2 on the Gut and Its Microbiome: What We Know and What We Would Like to Know

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ABSTRACT In the present review, we consider theoretical background and results of the first studies of SARS-CoV-2 effect on the intestine and its microbiome. The data obtained indicate the long-term virus persistence in the cells of intestinal mucosa. In addition, acceleration of microbial cells and microbial metabolites translocation associated with inflammatory processes in the intestinal endothelial cells caused by the virus was also discussed. COVID-19 has a great impact on structure and functional activity of the intestinal microbiome. The decrease in species diversity and minor species dominations that are not found in the microbiome of healthy controls were observed. The gut microbiome is considered to be an important influencer on COVID-19 progression and outcome.

Keywords: SARS-CoV-2, intestine, translocation, gut microbiome

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ACE2 - angiotensin converting enzyme 2
EndoCAb - endotoxin core antibody
ICU - intensive care unit
IgM - immunoglobulin M
I-FABP - serum intestinal fatty acid binding protein
TMPRSS2 - transmembrane serine protease 2

INTRODUCTION

Currently, the fact that the coronavirus infection COVID-19 induces a complex pathophysiological response of the entire human body is beyond doubt. A large number of publications are devoted to the study of changes in the respiratory, nervous, musculoskeletal, and immune systems of patients with COVID-19. The impact of SARS-CoV-2 on the intestines of patients has been studied to a lesser extent, mainly in the context of the development of gastrointestinal symptoms in a certain proportion of patients. The study of changes in the intestinal microbiome in patients with COVID-19 has so far been carried out on a very small number of patients.

Since the beginning of 2000, the intestine and its microbiome have been proposed to be considered as a special single organ that controls the functioning of other human organs and systems. The scientific literature has already included such concepts as “gut-brain axis”, “gut-lung axis”, implying the ability of intestinal microflora to synthesize many mediators with the help of which the functioning of the brain, lungs and other human organs and systems is regulated [1–3]. Microbial cells, their fragments and microbial metabolites enter the extraintestinal space, including as a result of direct translocation from the intestine. Until relatively recently, translocation was discussed based on the assumption of a very likelyendogenous source of bacteremia and sepsis in certain types of pathologies [4]. At present, quite convincing evidence of the reality of its existence has been

accumulated, although the mechanisms of its implementation have not yet been sufficiently studied.

In this review, we tried to discuss some of the theoretical background for the possible impact of the SARS-CoV-2 on the intestine and its microbiome, as well as to understand the real picture of events based on currently available data. It seemed very important to us to understand which changes in the intestinal microbiome are truly significant in order to use this knowledge in the future when developing measures to prevent severe course of COVID-19 disease.

A MYSTERIOUS INFECTION WITH MANY FACES

SARS-CoV-2 is an RNA virus and is part of the Betacoronavirus family [5]. Viruses of this family cause inflammatory diseases of the respiratory tract and gastroenteritis in animals and humans. In animals, coronaviruses can cause severe diarrhea with high mortality. The novel coronavirus infection caused by SARS-CoV-2 has raised a huge number of questions for the scientific, and most importantly, the practical medical community around the world. We were immediately struck by the incredible range of clinical scenarios for its course - from asymptomatic carriage to the rapid development of systemic inflammatory reactions ("cytokine storm"), with damage to the lungs, liver, kidneys, the development of thromboembolic complications, cardiovascular pathology, leading in a particularly unfavorable course of infection to multiple organ failure [4]. Even ultra-intensive resuscitation measures do not always stop the inflammatory cascade triggered by coronavirus [6]. Other problems include the frequent development of post-COVID complications, a long recovery period, and even the possible chronic course of the disease.

Such a different reaction of the human body to coronavirus is determined, first of all, by how effectively the initial links of the nonspecific antiviral immune response work, which are triggered within the first few hours after contact with the virus (for example, interferon synthesis). The further interaction of the body's nonspecific and adaptive immune response (the first two weeks) determines the success of containing the development of the infection, and its entry from the upper respiratory tract into the lungs, intestines, kidneys, and other organs [6]. At all stages of the adaptive response of the body's immune system to contact with the SARS-CoV-2, the gastrointestinal tract and its microbiota play the most active role in maintaining the so-called immunological homeostasis or the balance of pro- and anti-inflammatory intermediates, which allows events to develop in the "right direction" [8]. A number of researchers even suggest talking about the formation of a certain complex organism with a unified genome (the so-called "holobiont"), in which the human body and its microbiota form a continuous metabolic network [9]. But the point, of course, is not about terminological acrobatics. The special role of intestinal microflora (the term "microbiome" is now preferred) in the balanced functioning of the immune and other body systems is an indisputable fact.

COVID-19 AND THE GASTROINTESTINAL TRACT

The virus affects not only the lungs, although pneumonia is the main diagnosis in severe coronavirus infection. According to the results of several randomized studies, 9.8–20% of patients hospitalized with the diagnosis of COVID-19 develop gastrointestinal symptoms (diarrhea, nausea, pain) [10, 11]. The virus can disrupt the normal functioning of the vagus nerve and cause inflammation of the intestinal mucosa, which leads to the development of gastrointestinal symptoms [12]. However, the results obtained in a number of studies do not allow us to unambiguously answer the question to what extent gastrointestinal symptoms reflect the severity of the condition and the possible outcome of the disease [12–15].

The complex picture of the COVID-19 impact on the intestine and its microbiota is confirmed by the frequent unpredictability of manifestations of damage to the gastrointestinal tract. Moreover, these manifestations are observed both in the presence of viral particles in the intestine and in their absence. Already in the first months of the pandemic, reports appeared about the detection of the virus in the epithelial cells of the mucous membrane of the stomach, duodenum, small and large intestine [16–17]. Some researchers believe that the long-term existence of the viable virus in epithelial cells and in fecal matter is not associated with the severity of the condition, and rather indicates the dynamics of "self-cleaning of the body" [18]. It was proposed to take this fact into account in epidemiological terms, considering the additional possibility of spreading infection in

public places not only by airborne droplets, but also by the fecal-oral route. Subsequently, new evidence emerged of the possible existence of the fecal-oral route of COVID-19 transmission. This became obvious when examining young children who were found to be asymptomatic carriers of the virus, and often the virus was detected only in material taken with a rectal swab, and not from the nasopharynx [19].

The receptor for COVID-19 is angiotensin-converting enzyme 2 (ACE2). For the virus to attach to mucosal cells, a certain adaptation (priming) of its anchor protein is required, which is carried out by another protein synthesized in the human body—transmembrane serine protease 2 (TMPRSS2) [20, 21]. By means of immunohistochemical methods it was shown that the maximum ACE2 expression was characteristic for the gastrointestinal tract, and the minimum one – for the lung tissue [20]. Using various molecular methods (RNA-sequencing, single-cell transcriptomic analysis), a high level of TMPRSS2 expression was detected in the cells of the mucous membrane of the colon and small intestine, stomach, and lung tissue [22, 23]. These results make it surprising that gastrointestinal symptoms are not found in all patients with COVID-19.

Let us emphasize once again that the frequency of detection of actively reproducing viral particles in the epithelial cells of human mucous membranes varies widely (according to various studies, from 29 to 80%), while the duration of residence of the virus in the intestine can reach several weeks [24]. And we are talking about the long-term presence of viable viral particles [25, 26].

There are few works devoted to the dynamics of persistence of viral particles in the cells of the intestinal mucosa [27]. Thus, for 23 Chinese hospital patients in whom the virus was detected in 66.7% of nasal swabs and in 83.3% of rectal swabs, it was shown that the average peak values of the viral load in nasal swabs were reached by the 10th day (range of values: 8–17 days); while in rectal swabs, this peak was reached by the 22nd day (range of values: 15.5–23.5 days). Absolute peak values were 2535 and 5623 copies/mL for nasal and rectal swabs, respectively. The data obtained suggest that the inconsistent detection of the virus in one or another biotope is associated with a complex and dynamic picture of the persistence of the viral infection, which is manifested in the very varied clinical picture of COVID-19 in different people.

Trials, so far on a limited number of people, show clear signs of inflammation of the mucous membranes of the small and large intestines in patients with COVID-19. The detection of increased levels of calprotectin (a marker for acute inflammation, one of the cytosolic proteins of granulocytes) in the feces of patients, the level of which directly correlated with the level of IL-6 (interleukin-6) in the blood plasma, the authors of the study considered convincing evidence of inflammation of the intestinal mucosa [28, 29]. The virus was detected in the feces of all the patients examined, and diarrheal syndrome was found in just over half of the patients.

The inflammatory changes in the cells of the intestinal mucosa suggested a violation of their barrier functions and the very likely presence of translocation of intestinal microflora, fragments of microbial cells, and their metabolites into the extraintestinal space and into the systemic circulation. Normally, the dense architecture of the epithelial cells of the intestinal mucosa is a difficult barrier for microbial cells to overcome. But in the case of various disorders that occur in stressful situations for the body, for example, as a result of inflammatory processes, this barrier can be significantly weakened [30–32]. Oliva A. et al. [33] researched the translocation in patients with COVID-19 using 3 plasma biomarkers: lipopolysaccharide (LPS) binding protein (LBP), intestinal fatty acid binding protein (I-FABP) produced by enterocyte cells and a marker for enterocyte injury, and endogenous anti-endotoxin core antibodies (EndoCAb and IgM). The authors believe that a high level of all the three indicators is convincing evidence of the presence of translocation, and the intensity of translocation was directly related to the severity of the patient's condition. A high level of translocation was maintained for 7 days from the moment of admission to the hospital. It should be noted that 45% of the patients from the study group were in extremely serious condition and were treated in the intensive care unit (ICU). Interestingly, ICU patients had lower I-FABP (a marker for enterocyte damage) values than the clinical patients. Examination of the ICU patients who had bacteremia showed low EndoCab and IgM values. The main conclusion that was reached in this work was that COVID-19 significantly increased the permeability of the intestinal endothelium, even without visible

disruption of its structural integrity. As other biomarkers of translocation, a number of authors suggest using directly components of microbial cells—lipopolysaccharide (LPS) of gram-negative bacteria, and peptidoglycan (PGN) of gram-positive bacteria, as well as fatty acid binding protein 2 (FABP2) [34]. A twofold increase in the level of these indicators in 16 patients with COVID-19, compared with the control group, according to the researchers, convincingly indicated serious changes in the structure of epithelial cells of the intestinal mucosa and their increased permeability. Currently, the study of the effect of COVID-19 on the state of enterocytes is actively continuing, since it is quite obvious that increased translocation processes in COVID-19 patients are associated with a serious risk of developing secondary infections, since the intestinal microflora is a truly inexhaustible reservoir of various opportunistic microorganisms.

THE GUT MICROBIOME: THE SHADOW PLAYER

To assess the degree of influence of the SARS-CoV-2 on the intestinal microbiome, it is necessary to know, clearly and definitely, what constitutes its initially "normal" state. The idea of normobiocenoses (or normocenoses) has been "cultivated" in the scientific literature for many decades. Its use made it possible to maintain a broad perspective on many pressing issues of microbial ecology, interest in which increased due to the awareness of the fact that a forced "aggressively active" approach to treating patients is always associated with the risk of complications. But our knowledge about the microbiomes of various ecotopes of the human body is still very poor. Many studies of the intestinal microbiome in patients with COVID-19 are devoted to the traditional search for species of microorganisms that would indicate the presence of serious dysbiotic disorders. This simplified approach is, of course, forced. Rare attempts are being made to "structurize" the microflora based on the similarity of substrate preferences, physiological characteristics, occurrence in the same ecotopes, etc. [35, 36]. In that same vein, research is being conducted on the so-called "core" microbiome, which should be similar in structure and principles of functioning to healthy people. This approach is certainly promising, but it requires more knowledge about human microbiomes.

In order to assess the extent of dysbiotic changes in patients with COVID-19, it is worth turning to some basic principles of microbial ecology. According to the postulates declared within the framework of general population ecology, any mature biological system maintains its stability due to large species diversity; and any shift towards a decrease in this diversity due to the dominance of one or another species or group of species is a sign of instability, which in the future can lead to the collapse of the system [37]. Consequently, the level of dysbiotic disorders can be assessed by the degree of reduction in the species diversity of the intestinal microflora, which, as already shown by the first trials, is observed in patients with COVID-19 [38–40]. In addition to the decrease in species diversity, there is also a dominance of bacterial species that belong to the minor group in healthy people (almost never encountered species), primarily an increase in the proportion of gram-negative bacteria, and a decrease in the number of gram-positive bacteria [40]. Interestingly, similar structural changes are observed in the microflora of the respiratory tract, which brings to mind the "gut-lung axis", the functioning of which allows synchronizing the metabolic activity of two biotopes (intestine and lungs) [41–44]. This relationship, or better said, the bidirectionality of regulatory mechanisms, has often been discussed in the study of the pathophysiological processes underlying obstructive pulmonary disease and inflammatory bowel diseases [42]. A kind of closed loop develops when inflammatory processes in the lungs caused by the virus change the intestinal microbiome, what, in turn, leads to a shift in the metabolic profile of the intestinal microflora, which is the driver of a new round of increased synthesis of pro-inflammatory mediators in the lungs, etc. [43]. Moreover, a number of authors make the bold assumption that the initial state of the intestinal and lung microbiomes before the onset of the disease largely determines the clinical picture of the viral infection and its outcome [44–46].

The human gastrointestinal tract is the most important part of the human immune system (it contains up to 70% of lymphoid tissue), a kind of regulatory center that ensures the maintenance of homeostasis of the immune system through thesynthesis of regulatory mediators [36]. Therefore, it seems appropriate to take the basic

("normal") metabolic profile of the intestinal contents of a conditionally healthy person as a starting point, and to it already link those iconic (or indicator) species information about which we currently have. Little has been known yet about changes in the metabolic profile of the intestines of patients with COVID-19. It was shown on a limited number of patients that significant downward shifts are observed in lipid metabolism and glycan biosynthesis, while the metabolism of simple carbohydrates is accelerated [41, 44]. One pilot trial examining 45 fecal metabolites that were significantly altered in COVID-19 patients found that most of these metabolites were involved in the biosynthesis of amino acids, proteins, fatty acids and bile acids [47].

The search for indicator microorganisms which could be used to judge the degree of dysbiotic disorders, has been going on for several decades. It is difficult to say how productive this approach is, since knowledge on how human microbiomes (not only intestinal, but also others) actually function is still clearly insufficient. It seems that formulations such as "opportunistic pathogens" or "beneficial commensals" are rather vague. For example, the group of "harmful" microorganisms includes *Clostridium hathewayi*, *Actinomyces viscosus*, *Bacteroides nordii*, *Streptococcus spp.*, *Rothia spp.*, *Veillonella spp.*, *Erysipelatoclostridium spp.*, and the group of useful ones includes *Faecalibacterium prausnitzii*, bacteria of the *Lachnospiraceae* family (*Fusicatenibacter*, *Anaerostipes*, *Agathobacter*, *Blatia*, unclassified *Lachnospiraceae* and *Eubacterium hallii* group), *Eubacterium rectale*, *Ruminococcus spp.*, *Dorea formicigenerans* [39–41, 48]. It is noteworthy that the systematic position of some "useful" species is constantly being revised, and their new generic and species names may not be final. Thus, *Dorea* "branched off" from the genus of *Eubacterium* [49], and *Blatia* previously belonged to the genus of *Ruminococcus* [50]. It is significant that the concept of "rRNA group of organisms" has come into scientific use, since the taxonomic position of the members of this group, or "genomic species groups", has not yet been fully established, and there is no transition to real groups yet.

Despite the well-known skepticism regarding the identification of groups of "useful" and "harmful" microorganisms, it is worth recognizing that the "usefulness" of representatives of this group is based on the following completely objective characteristics. Firstly, they are part of the so-called "core" gut microbiome, which currently comprises about 10 genera, with each of these genera being isolated from the feces of healthy people in 2–8% of cases in different variations [51, 52]. Secondly, the ability of these species to produce short-chain fatty acids (butyrate, propionate, acetate, succinate) was shown. Short-chain fatty acids are not only necessary substrates for enterocytes and glial cells that surround neurons and ensure reliable transmission of nerve impulses, but they also play the role of signaling metabolites that carry out mutual regulation of various human systems and organs. Moreover, fatty acids are universal regulators necessary to maintain the homeostasis of the intestinal microbiome itself [53, 54]. As for "harmful" microorganisms, not everything is so certain and clear. Metabolically, this group is more diverse. The only quality that unites all the "harmful" species is their variable and rare occurrence in the healthy part of humanity. It would seem that this sign is quite convincing, but the frequency of detection largely depends on the technical capabilities of researchers. Therefore, relying only on detection rates seems insufficient. The most convincing candidates for the role of "harmful" microorganisms are representatives of fungal microflora (*Candida albicans*, *Candida auris*, *Aspergillus flavus*) [48, 51], since a number of large randomized trials have shown that in practically healthy people these types of micromycetes are found extremely rarely [48, 51].

Let us emphasize once again that a possible solution to the problem of assessing the degree of dysbiotic disorders at the present stage may be the use of the microbiome diversity index [56, 57].

One cannot ignore the issue of multicomponent therapy effect on the intestinal microbiome. This is especially true for patients with COVID-19, since the infection affects almost the entire body. Most often, and quite understandably, we are talking about the effect of antibiotic therapy. But it is quite obvious that other therapeutic agents cannot but influence, to one or another degree. There are very few studies on the impact of "polypharmacy" [57–59]. In these studies which were conducted back in the "pre-COVID era" it was found that during the treatment for cardiovascular diseases in elderly patients, the number of bacteria of the *Lachnospiraceae*, *Succinivibrionaceae* families ("useful" butyrate producers) decreased, the

incidence of isolation of *Helicobacter pylori* increased, and the microbiome diversity index was significantly reduced [57, 58]. Moreover, the values of the microbiome diversity index were inversely proportional to the number of drugs administered. In patients with fatal outcomes, the values of this index were extremely low [57]. It was shown that, in addition to antibiotics, proton pump inhibitors of intestinal mucosal cell membranes, antacids, laxatives, statins, metformin, and psychotropic drugs had an undoubted effect on the intestinal microbiome [58]. Glucocorticoids also had a visible effect on the gut microbiome, increasing the number of gram-positive bacteria (*Firmicutes*) and decreasing the number of gram-negative bacteria (*Bacteroidetes*) [60].

Particular mention should be made of the use of broad-spectrum antibiotics for therapeutic (and even prophylactic!) purposes in the treatment for COVID-19. First of all, we are talking about azithromycin, which was used prophylactically in many countries during the first wave of COVID-19, and this not only stimulated the growth of strains resistant to it, but also caused considerable harm to the intestinal microbiome, since, as it turned out, its use led to a sharp decrease in the species diversity [59]. A number of researchers believe that the use of antibiotics for prophylactic purposes could contribute to the progression of COVID-19 in a more severe scenario [59, 60].

WHAT CAN BE OFFERED TO MAINTAIN A HEALTHY GUT MICROBIOME?

No breakthroughs can be expected on this issue yet. A possible solution may be the old proven remedies - preventive use of probiotics, eubiotics and a diet rich in plant fibers, which ensures the growth of microorganisms that produce short-chain fatty acids. This approach has been used for many years in the treatment of inflammatory diseases of the gastrointestinal tract.

The immunomodulatory effect of pro- and eubiotics has long been undisputed not only by the scientific community, but also by the practical medical community [59, 61, 62]. Their anti-inflammatory effect is especially emphasized, as well as the positive effect on enterocytes (restoration of their barrier functions). Work carried out by Italian researchers showed that the inclusion of a drug containing cells of lactobacilli and bifidobacteria into the standard treatment regimen of 28 patients of moderate severity (three times over two weeks) led to the fact that the disease proceeded according to a milder scenario (none of the patients were transferred to the ICU, there were no deaths), in contrast to the control group of patients [63]. In another study [64], the same drug was used in a larger group of patients (200 people). It turned out that three-time administration of probiotics for two weeks along with the main therapy allowed a 2-fold reduction in mortality (11 versus 22%). Similar trials are currently being conducted in many countries (Mexico, Canada, Spain), but the results of these studies have not yet been published [59].

CONCLUSION

Since the very beginning of the COVID-19 pandemic, there has been evidence of undoubted damage to almost all human organs and systems, including the gastrointestinal tract. Long-term persistence of the virus in the cells of the intestinal mucosa, even after recovery, was discovered, which gives some researchers reason to assume the possibility of chronic infection.

An increase in the processes of translocation of microbial cells and their metabolites was revealed, which is most likely associated with inflammatory processes in the cells of the intestinal mucosa caused by the virus. Changes in the state of endothelial cells of the intestinal mucosa naturally affect the composition and functional activity of its microbiome. The research results obtained have not yet been sufficiently systematized. The human intestinal microbiome is still a “black box”, the functionality of which we judge by how it reacts to one or another external influence.

Since the beginning of the study of microbiomes of various human biotopes in the early 2000s, the avalanche-like flow of information splashed out into the global scientific space has caused incredible euphoria, followed by the sobering realization that the main difficulty lies in the ability to “process” this information. The COVID-19 pandemic once again sharply reminded us of the need for this systematization.

It is an undoubted fact that the role of the intestinal microbiome can be considered if not decisive then at least very important for the development of COVID-19. It is clear that in patients with COVID-19, the structure and functional activity of the gut microbiome changes

greatly during the course of the disease. There is a decrease in species diversity, and a dominance of species that are not typical for the microbiomes of healthy people is noted. However, the currently available data is clearly insufficient to draw any definitive conclusions. The scatter of data on the composition of microflora is so great that it is still not possible to talk about general patterns. New trials are required with patients of homogeneous groups, not only of moderate severity (most studies were conducted with this group of patients), but with the involvement of severe patients.

It is too early to talk about indicator species of microorganisms, by the presence or absence of which we could judge the degree of dysbiotic disorders of the intestinal microbiome, although we should not give up trying to search for them. More promising, however, seems to be the further development of the concept of a "healthy metabolic profile" (or "core profile"), to which data on the microbial spectrum can be "linked".

Tracking the degree of changes in metabolic and microbial profiles will provide objective information that can be used to prevent coronavirus disease. It has still remained unclear how much the initial "pre-COVID" state of the human intestinal microbiome influences the course and outcome of COVID-19. The answer to this question is very important, since it largely determines the answers to other questions, for example, when and to what extent it is worth correcting the dysbiotic disorders that arise. The results of the first, still rare attempts to introduce pro- and eubiotics into the treatment regimen, as well as special diets, allow us to be optimistic about their use in the future.

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