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Microbiota of the gastrointestinal tract. Friend or foe?

Microbiota of the gastrointestinal tract

Abstract

The gut microbiota is currently considered an external organ of the human body that provides important mechanisms of metabolic regulation and protection. The gut microbiota encodes over 3 million genes, which is approximately 150 times more than the total number of genes present in the human genome. Changes in the qualitative and quantitative composition of the microbiome lead to disruption in the synthesis of key bacterial metabolites, changes in intestinal barrier function, and inflammation and can cause the development of a wide variety of diseases, such as diabetes, obesity, gastrointestinal disorders, cardiovascular issues, neurological disorders and oncological concerns. In this review, I consider issues related to the role of the microbiome in the regulation of intestinal barrier function, its influence on physiological and pathological processes occurring in the body, and potential new therapeutic strategies aimed at restoring the gut microbiome. Herewith, it is important to understand that the gut microbiota and human body should be considered as a single biological system, where change of one element will inevitably affect its other components. Thus, the study of the impact of the intestinal microbiota on health should be considered only taking into account numerous factors, the role of which has not yet been fully elucidated.

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Core Tip: The gut microbiota affects the development and functioning of all body systems, providing metabolic, physiological, regulatory and protective functions. Violations in the qualitative and quantitative composition of the microbiome lead to the development of a wide variety of diseases, such as diabetes, obesity, cardiovascular issues, neurological disorders and oncological concerns. Considering that intestinal dysbiosis plays a key role in the development of a number of diseases, aim to normalize the microbiome seems to be a greatly perspective direction in their prevention and treatment

INTRODUCTION

Trillions of microorganisms, known as microbiota, colonize the human body. The human gastrointestinal tract harbours more than 1000 species of bacteria belonging to a relatively few known bacterial phyla[1]. Features of their mutual coexistence determine the nature of various physiological and pathological processes occurring in the human body. To discuss these issues, a search was made in the PubMed database (https://pubmed.ncbi.nlm.nih.gov/) and Reference Citation Analysis (https://www.referencecitationanalysis.com/) for studies published up to July 1st, 2022 using a combination of text keywords "gut microbiota", "bacterial metabolites", "intestinal barrier", "dysbiosis", "fecal microbiota transplantation", "probiotics". A total of 846 unique results were identified, which were screened individually by title and abstract and were included based on the role of the microbiome in the regulation of intestinal barrier function, its influence on physiological and pathological processes occurring in the body, and new therapeutic strategies aimed at restoring the gut microbiome.

It is believed that bacteria begin to colonize the human intestine immediately after birth and, possibly, even in utero[2,3]. Breast milk plays a crucial role in the composition of the

microbial community within newborns through transfer of the milk microbiota to the infant's gut[4]. In the first 6 mo of a child's life, there is a steady increase in the number of *Enterobacteriaceae*, *Bifidobacteriaceae* and *Clostridiaceae*. However, the microbiome of children differs depending on diet, gender, race and ethnicity[5,6]. In addition, the mode of delivery may affect the composition of the gut microbiota of early infants[3,7]. In particular, in children born by cesarean section, there is a high abundance of *Bifidobacterium,* and *Clostridium* genera and the family Enterobacteriaceae, along with a low abundance of *Streptococcus* and *Ruminococcus* genera[8]. Moreover, in children born by cesarean section, the *Bacteroides* genus is not detected in the feces until 6–18 mo after birth[9].

Microorganisms living in the gut of adults include bacteria, fungi, protozoa, archaea, and some viruses[10]. The total number of bacteria in a 70 kg "reference man" is estimated at 3.8×10^{13} cells, which is comparable to the number of human host cells (3.0×10^{13})[11]. The specific microbiota at the genus and species levels varies depending on geography, environment, diet, age, genotype, presence of diseases and lifestyle[12,13]. For instance, if the prevalence of proteins and animal fats in the diet exists, *Bacteroides* will predominate in the microbiota and if there is a high level of carbohydrates, then *Prevotella* will ascendant. The gut microbiota encodes over 3 million genes, which is around 150 times more than the number of genes in the host genome[14]. Approximately 90% of the composition of the gut microbiome is represented by *Firmicutes* (79.4%), *Bacteroidetes* (16.9%), *Actinobacteria* (2.5%) and *Proteobacteria* (1%)[5,15]. The number of microorganisms increases from the proximal to the distal gastrointestinal tract and from the epithelial layer to the lumen. This difference can be explained by the presence of a more aggressive environment in the upper intestines due to the incoming gastric acid, action of digestive enzymes, rapid movement of chyme and the decrease in partial pressure of oxygen in the distal gastrointestinal tract. For this reason, aerobic bacteria predominate in the small intestine, while facultative and obligate anaerobes predominate in the lower gastrointestinal tract. Furthermore, the distribution and organization of the gut microbiota is determined by intestinal mucins, which protect

intestinal epithelial cells from bacterial colonization. At the same time, the presence of the gut microbiota is a necessary condition for normal functioning of the mucosal barrier[16]. For example, mice treated with antibiotics had a thinner layer of mucus[17,18].

GUT MICROBIOTA AND INTESTINAL BARRIER

Partition of the body's internal environment from the intestinal microbiota is carried out by three types of barriers: physical, chemical and immunological one. The physical barrier consists of epithelial cells, glycocalyx and a layer of mucus covering the surface of the gastrointestinal wall[19].

Physical barrier

The intestinal epithelium is the most rapidly self-renewing tissue in adult mammals. To maintain epithelial layer integrity, intestinal epithelial cells (IECs) are continuously replaced by proliferating progenitor cells, derived from multipotent intestinal stem cells (ISCs) localized in the base of the crypts of Lieberkühn and the colon[20]. IECs differ in their proliferation ability, renewal rate and age. Aged IECs undergo apoptosis and are later ejected into the intestinal lumen, whereas Paneth cells leave the crypt bottom by cellular fragmentation and phagocytosis by macrophages infiltrating the lamina propria mucosae[21]. Under homeostatic conditions, the entire ileal crypt is replaced every 4–5 days[22].

It is customary to distinguish the following populations of the intestinal epithelium[22-24].

1. *Columnar cells* (colonocytes) are the most numerous population of enterocytes. They participate in digestion due to the secretion of digestive enzymes, the absorption of digested products and transcellular transfer of dissociated monomers into the blood and lymph and take part in the exchange of bile acids and humoral immune response. Absorbent enterocytes produce polymeric immunoglobulin receptor (pIgR), which mediates transcytosis of dimeric IgA and polymeric IgM from the lamina propria through the epithelial barrier to the mucosal surface, ensuring the binding of bacteria and viruses on their surface and thereby preventing the penetration of pathogens into

the internal environment of the body. In addition, during transcytosis of IgA through the epithelium, it can neutralize viruses that have entered cells, as well as bind and excrete proteins and other immune complexes on the surface of mucous[20,25];

2. *Goblet cells* are a source of mucus. In addition, they can deliver small soluble antigens to dendritic cells localized in the lamina propria and thus participate in the formation of immune tolerance to food antigens and the gut microbiome[20,26];

3. *Enteroendocrine cells* secrete peptides and hormones (cholecystokinin, serotonin) to stimulate intestinal motility;

4. *Tuft cells* participate in the clearance of parasites from the intestinal lumen due to the synthesis of IL-25, which is the key activator of type 2 innate lymphoid cells;

5. *Paneth cells* in the small intestine or deep crypt secretory cells in the large intestine are the main regulators of microbial density in the intestines. When interacting with gram-negative bacteria, gram-positive bacteria or their products (lipopolysaccharides, lipoteichoic acids, lipid A, muramyl peptide), they secrete antimicrobial peptides[20,27,28]. Moreover, Paneth cells secrete important factors such as epidermal growth factor, transforming growth factor-α, and Wnt ligands involved in stem cell maintenance[29];

6. *Microfold cells (M)* are located in the follicle-associated epithelium (FAE) overlying Peyer's patches and stimulate an immune response by binding luminal antigens for their further transport to subepithelial regions, where they are captured by dendritic cells (DCs) migrating to the mesenteric lymph nodes and stimulating the immune response[20]. The interaction of DCs with T cells stimulates an antigen-specific immune response directed against the pathogen or, conversely, leads to the induction of tolerance. Activation of B cells leads to the secretion of IgA, which plays an important role in the regulation of the gut microbiome. Immunoglobulin A, on the one hand, is involved in the binding and elimination of pathogenic bacteria, and on the other hand, it facilitates the translocation of commensals into Peyer's patches, activating the mechanisms of immunological tolerance and thereby stimulating the growth of intestinal symbionts[30,31].

It is worth noting that on the level of IECs, a structure composed of three junctions is formed: tight junctions (TJs), adherens junctions (AJs) and desmosomes [20,32-34]. They provide mechanical strength between cells, intercellular adhesion and polarization and are also involved in cell signaling pathways[19]. The strength of the mechanical barrier also depends on the regeneration rate of the intestinal epithelium. It was established that the presence of intestinal microorganisms affects the number of Paneth cells and hence the integrity of the epithelial barrier, as Paneth cells regulate stem cell homeostasis[35]. Bacteria can directly damage tight junction proteins or interfere with their synthesis *via* type 3 (T3SS) or type 4 (T4SS) secretion systems. The disruption of cell contacts can also be mediated by some bacterial enzymes and toxins, such as hemagglutinin/protease and ZO toxin, as well as bacterial metabolites, such as ethanol and acetaldehyde[13,36]. Apart from that, the gut microbiota can alter the mitochondrial metabolism of epithelial and immune cells, thereby activating inflammation and disrupting epithelial barrier function[37]. Integrity violation of the intestinal epithelium, the mucus barrier and cell contacts leads to the translocation of bacteria[38].

Recognition of the bacterial microbiota is carried out by TLR and NOD receptors, which are expressed by most IECs, including stem cells, enterocytes, goblet cells, enteroendocrine cells, Paneth cells, and M cells[19]. Most TLRs (TLR2, TLR3, TLR4, TLR5, and TLR9) are present on the basolateral membrane, while TLR2, TLR3, and TLR9 are also expressed on the apical surface. TLR5 expression is limited to Paneth cells in the epithelium of the small intestine and proximal colon[39,40]. Activation of innate immune system receptors, including TLR and NOD receptors, as well as inflammasomes, leads to a signaling cascade that triggers the secretion of cytokines and chemokines, including IL-1β, IL-6, IL-12, IL-18, TNF-α, CXCL8 and CCL20, which activate immunocompetent cells localized in the lamina propria[41-43]. Mitochondrial reactive oxygen species (mtROS), produced by immune cells, play a key role in the eradication of invading pathogens through direct bactericidal action or indirect impact on the activation of NLRP3 inflammasome and the production of proinflammatory cytokines. Invading bacteria, as well as gut microbiome fermentation products such as

short-chain fatty acids (SCFAs), induce mtROS production in immune cells through increased mitochondrial respiration and increased oxidative phosphorylation[44]. Hypoxia inducible factor-1α (HIF-1α) is believed to be the main regulator of mitochondrial responses during bacterial infection[37]. At the same time, damage to the intestinal epithelium mitochondria by toxins of pathogenic bacteria leads to the accumulation of mtROS and disruption of the barrier function of the epithelium[45,46].

Intestinal mucus (mucins). Intestinal mucus is the first barrier for microorganisms in the gastrointestinal tract. It regulates nutrient and drug delivery, regulates symbiosis with the gut microbiota and protects the epithelium from dietary antigens and food toxins[20,47,48]. The thickness of the mucus layer in the small and large intestine is not the same. The main function of the small intestine is food digestion and absorption of nutrients, so the small intestine has a loose, discontinuous layer of mucus that can be easily removed. In the large intestine, where the density of microorganisms is much higher than in the small intestine, the number of mucus-producing goblet cells is significantly larger[20]. In the large intestine, the mucus layer is organized into two layers: an inner, dense, microbiota-free mucus layer and an outer layer, which is friable and permeable for microorganisms[13,17,31,49,50].

More than 20 mucin subtypes have been identified in humans. The best known and most studied one, found in the small and large intestines, is MUC2[17,49]. MUC2 is a highly O-glycosylated gelling mucin that forms polymeric networks *via* C-terminal dimerization and N-terminal trimerization. MUC2 monomers are glycosylated in proline, threonine, and serine (PTS)-rich domains[51,52].

Mucus is secreted by goblet cells, grows rapidly and forms a stratified, dense layer that adheres to the epithelium[53]. On the one hand, mucus is necessary for the normal functioning of the gut microbiome, but on the other hand, the presence of the microbiome is a necessary condition for the normal functioning of the mucus barrier[54]. As noted above, mice treated with antibiotics have a thinner layer of mucus[17,18].

The barrier function of the mucus is confirmed by the fact that mice genetically deficient in Muc2 (Muc2 -/-) have bacteria invading the normally sterile distal colon crypts,

which results in the development of spontaneous colitis[55], adenomas arising in the small intestine and an invasive cancer[56]. However, intestinal bacteria can directly influence the production and quality of intestinal mucus and hence the intestinal barrier permeability[57]. Bacteria and their metabolites that enter crypts are endocytosed by specialized goblet cells known as 'sentinel' goblet cells. This leads to the activation of TLR2/1, TLR4, and TLR5 Ligands with activated ROS synthesis, triggering the formation of the NLRP6 inflammasome and Ca2+-dependent compound exocytosis of MUC2-containing granules[58,59]. Importantly, increased regulatory secretion leads to the secretion of large amounts of MUC2 and the physical removal of bacteria from the crypt opening, thereby protecting the inferior crypt and multipotent ISCs, located at the bottom of the crypts, from bacterial invasion[20, 60].

Chemical barrier

An important function of the chemical barrier is to maintain the abundance and composition of the gut microbiome. The chemical barrier includes antimicrobial peptides (AMPs), gastric acid, digestive enzymes, mucopolysaccharides, glycoproteins, glycolipids, and other compounds[19,61,62]. In addition, the composition of the microbiota can be influenced by various factors such as hygiene, diet (especially the "Western diet" low in fiber and high in sugar and fat), oxygen concentration, microbial adhesion, host stress and other factors[17,49,63,64]. It is believed that microbiome regulation in the small intestine is mainly carried out by antibacterial peptides, while in the large intestine this regulation is carried out through pattern recognition receptors[5]. The microbiome population is maintained, either by preventing colonization or through direct killing mechanisms.

The production of antimicrobials that lyse target cells is one of the main mechanisms for regulating the homeostasis of the gut microbiome. The contact of bacteria with Toll (TLR2, TLR4, TLR7 and TLR9), NOD1 and NOD2 receptors activates adapter proteins (for example, MyD88) and genes responsible for the synthesis of cytokines and chemokines in IECs[65-68]. In turn, the synthesis of cytokines by immune cells activates the genes responsible for the synthesis of antimicrobial peptides[69]. Thus, mice deficient

in MyD88 exhibit a 100-fold higher bacterial load in the gut than wild-type mice and this increase is correlated with a decrease in the antibacterial peptide RegIIIgγ[70]. Paneth cells expressing TLR5 are major producers of antimicrobials, many of which are cationic antimicrobial peptides that interact with negatively charged bacterial membranes and destroy them[12]. Interestingly, TLR5 expression occurs predominantly in intestinal crypts and is genetically determined, as TLR5 expression does not require bacterial or immune signals. It is believed that their main function may be related to the protection of Paneth cells and stem cells[31,71].

Other antimicrobial peptides are also involved in the regulation of the gut microbiome population. For example, protein 8 (Lypd8) is highly expressed by colonocytes and facilitates segregation of microorganisms in the colon *via* flagella binding[61]. The lectin-like protein ZG16 specifically binds the peptidoglycan of gram-positive bacteria and thereby inhibits their penetration into the inner layer of the colon[62]. Lectins RegIIIγ from intestinal epithelial cells and beta-defensins from neutrophils have a bactericidal effect against a number of bacteria. However, *Firmicutes* and *Bacteroidetes* living in the small intestine are resistant to these antibacterial agents[72]. It is also worth noting that some bacteria can synthesize bacteriocins (for example, colicin and microcins), which inhibit the growth of competitors[73,74].

Immunological barrier

The gut immune barrier is represented by single lymphoid follicles and Peyer's patches - peripheral accumulations of lymphoid cells located in the lamina propria of the small intestine mucosa[20]. Within the follicles there are various immune elements, including B and T lymphocytes, DCs, and neutrophils that secrete cytokines and antibodies in response to antigen entry. Goblet cells are involved in the presentation of luminal antigens to the CD103+ DCs complex of the intestinal mucosa lamina propria, forming antigenic complexes (goblet cell-associated antigen passages, GAPs)[75]. Secretory IgA (SIgA), another component of the intestinal barrier, is produced by plasma cells (50 mg/kg SIgA daily in an adult) and localized predominantly in the lamina propria of the intestinal mucosa[31]. It is believed that SIgA is able to interact with commensal

intestinal bacteria, mediating the formation of a bacterial biofilm. SIgA is resistant to the action of intestinal proteases, which provide protection for bacteria. SIgA can penetrate through the epithelial lining into the intestinal lumen, bind antigens and deliver them to the immune cells of the lymphoid tissue[76].

Elements of immune protection can also include an increase in "tolerance" to a microbe (or toxin of microbial origin)[52] and the death of infected cells. In particular, flagellins of pathogenic bacteria that have overcome the epithelial barrier are able to activate NAIP/NLRC4 in macrophages, which causes the death of infected epithelial cells and their expulsion into the intestinal lumen[77].

It is important to note that the intestine is the most important immune organ, which not only protects against external pathogens but also participates in the formation of immune tolerance to food substrates and the normal gut microbiome. The main cytokines involved in the formation of immunological tolerance are IL-10 and TGF-beta, which are produced by CD4+ T cells, some populations of macrophages and other cells and have an anti-inflammatory effect, limiting the expansion of effector cells and inducing the proliferation of regulatory T cells[78].

FUNCTIONS OF THE GUT MICROBIOTA AS AN EXTERNAL ORGAN OF THE HUMAN BODY

The microbiota are currently considered an external organ of the human body, which provides important mechanisms of metabolic regulation and protection, alongside the development and functioning of all organ systems[79]. It performs the following functions, the list of which is incomplete:

- Digestion of plant polysaccharides. Approximately 17 carbohydrate-active enzymes are formed in the human body, while the microbiota provides around 89[80]. The gut microbiota actively digests dietary fiber, which the human body is unable to digest. These processes take place in the large intestine *via* the most actively involved enzymatic anaerobes, which decompose polysaccharides, particularly representatives of the *Bacteroidaceae* and *Clostridiaceae* families[12,81]. As a result of their digestion,

compounds are produced that have a positive effect on the intestinal mucosa. In addition, the mucus layer is an alternative source of glycans for bacteria[12,51,81,82];

- Participation in the metabolism of proteins, lipids and fatty acids[83-87]. In particular, gram-negative (*Bacteroides thetaiotamicron*) and gram-positive (*Lactobacillus rhamnosus gg*) bacteria are involved in the regulation of lipid absorption by activating cholecystokinin (CCK) and secretin (SCT) receptors expressed by epithelial endocrine cells of the proximal small intestine[88];

- Energy supply of IECs[15,81,89] and regulation of their proliferative activity[90,91];

- Modulation of goblet cell functions and mucin secretion[16];

- The presence of intestinal microorganisms affects the number of Paneth cells and hence the integrity of the epithelial barrier, as Paneth cells regulate ISC homeostasis[35];

- Stimulation of local and systemic immunity due to activation of the synthesis of IgA, interferons, activation of immune cells (macrophages, lymphocytes, dendritic cells), influence on the development of the intestinal lymphoid apparatus in newborns[92-94];

- Synthesis of group B, K vitamins, a number of coenzymes, for example, tocopherols[95-97];

- Participation in the regulation of intestinal peristalsis[98-100];

- Influence on bone metabolism and pathogenesis of osteoporosis[14,101,102]. The bacterial synthesis of SCFA leads to a decrease in pH in the intestinal lumen and an increase in calcium solubility, an increase in its absorption, and a decrease in bone resorption. *Bifidobacterium* and *Lactobacillus* are mainly involved in these processes. In addition, F. nucleatum can enhance osteoclast differentiation through increased expression of IL-17A, TNF-alpha, and trimethylamine N-oxide, while *Bacteroides*, *Lactobacillus*, and *Bifidobacterium* can promote the development of Treg cells and thereby increase osteoblast activity[14,101,103,104];

- Influence on the processes associated with the synthesis of neurotransmitters, myelination of neurons in the prefrontal cortex, with the development of the amygdala and hippocampus[105,106]. In dysbacteriosis, the response to antidepressant therapy may be impaired[107]. Germ-free mice show hyperactivity, memory and learning deficits and

impaired expression of the serotonin 5-HT1A and NMDA receptors in the hippocampus[108,109];

- Inhibition of the growth of pathogenic microorganisms due to the activation of phagocytosis, the synthesis of antibacterial peptides or the synthesis of bacteriocins that inhibit the growth of competitors[69,73,74,81,100,110];

- Influence the effectiveness of several drugs, in particular antibiotics, proton pump inhibitors, metformin, vitamin D and laxatives. It has been shown that the use of these drugs disrupts both the composition of the microbiota and its functional activity[89,111,112].

PRODUCTS OF BACTERIAL METABOLISM AND THEIR ROLE IN HEALTH AND DISEASE

As already noted, health and disease conditions are largely dependent on the functioning microbiome. Products of bacterial metabolism can be crucial for maintaining both the health of an organism and the development of various diseases[113].

Short-chain fatty acids (SCFAs)

The most important products of bacterial fermentation are SCFAs: butyrate, acetate and propionate. The main producers of SCFAs are *Firmicutes* and *Actinobacteria*.

Butyrate is the primary metabolite of *Firmicutes*. It can be synthesized through condensation of 2 molecules of acetyl-CoA, which are reduced to butyryl-CoA and then converted to butyric acid by phosphotransferase and butyrate kinase[114]. It can also be synthesized from butyryl-CoA, lactate and acetate using the acetyl-CoA transferase pathway[115] and from proteins using lysine[116]. It has anti-inflammatory, antitumour, antiproliferative and immunomodulatory properties and is involved in genetic/epigenetic regulation[117,118]. In particular:

- Regulates antigen-specific adaptive immunity mediated by T- and B-cells: induce T-cells to produce IL-10; regulate the transcription of some cytokine genes, such as IFN-γ and TNF-α, and the activity of the NF-κB signaling pathway; reduce the production of

proinflammatory mediators (TNF-α, IL-6, IFN-γ and NO); increase the production of antibodies by B-cells and promote the differentiation of B-cells into plasma B-cells [119-121];

- Participates in fat metabolism, reduces insulin resistance, hyperglycaemia, hyperinsulinaemia, and lipid concentrations in the liver and pancreas, thereby reducing the risk of obesity [122];

- Affects fatty acid receptors in epithelial, enteroendocrine, neuronal and glial cells, which leads to the production of serotonin by enterochromaffin cells. This may affect the peripheral and central nervous systems of experimental model organisms[50,123];

- Helps improve memory[124];

- Involved in maintaining the mechanical integrity of the intestinal barrier by inducing the expression of occludin, ZO-1 mRNA and claudin-1 mRNA, thereby reducing intestinal permeability and increasing intestinal villus growth[13,122,125,126];

- Inhibits the rate of cancer cell migration and invasion by increasing the expression of antimetastatic genes (e.g., metalloproteinases) and inhibiting the activation of prometastatic genes (e.g., matrix metalloproteinases)[85,127].

Acetate is a fermentation product of various bacteria and is produced from pyruvate using acetyl coenzyme A[128]. It performs the following functions:

- Participates in the regulation of cholesterol synthesis and activation of local immunity[129];

- Helps increasing physical endurance[130];

- Influences cognitive functions by activating synaptophysin synthesis[131].

- Promotes appetite reduction, fat oxidation, increased levels of proinflammatory cytokines through activated secretion of intestinal hormones such as glucagon-like peptide-1 and peptide YY and increased insulin sensitivity[86]. Nonobese patients show higher production of acetate by gut microbiota than obese patients[132];

- Regulates the gut microbiome by increasing the production of IgA and its selective binding to certain microorganisms[86];

Propionate is the primary metabolite of *Bacteroidetes* fermentation and is formed from the conversion of succinate to methylmalonyl-CoA by the succinate pathway or from acrylate by the acrylate pathway using lactate as a precursor[133]. Additionally, fucose and rhamnose can be used as substrates for the synthesis of propionic acid *via* the propanediol pathway[134]. Propionate performs the following functions:

- Participates in maintaining the mechanical integrity of the intestinal barrier by increasing the expression of gut tight junction proteins ZO-1, occludin and cadherin[135,136], as well as the synthesis of the antimicrobial protein Regenerating islet-derived protein type 3 (Reg3)[137];

- Reduces the risk of developing atherosclerosis and the development of cardiovascular disease by increasing insulin sensitivity and reducing the levels of proinflammatory IL-8[138] and IL-17[139], as well as by reducing the absorption of cholesterol in the intestine by an immune-mediated mechanism through an increase in the number of regulatory T cells and the level of interleukin (IL)-10, which suppress the expression of C1-like 1 Niemann-Pick (Npc1 L1), the main cholesterol transporter in the intestine[140].

- Influences physical endurance[141] and motor functions[136];

- Promotes regeneration and functional recovery of sensory axons through an immune-mediated mechanism[142].

- Involved in the regulation of the gut microbiome, possibly through direct suppression of the growth of pathogenic microorganisms[143].

Tryptophan derivatives

A number of other intestinal metabolites can also affect the health of individuals. Among these metabolites, a special place is occupied by tryptophan derivatives: serotonin, tryptamine, kynurenine, and indoles. They perform the following functions:

- Act on the central nervous system through the brain-gut axis[144]. The influence is implemented due to the impact on the glutamatergic receptor N-methyl-d-aspartate (NMDA). It has been established that kynurenine, a breakdown product of tryptophan, easily penetrates the blood–brain barrier, where it is metabolized to form neuroactive glutamatergic compounds, kynurenic acid or quinoline acid, acting in the opposite way.

Kynurenic acid acts as an NMDA receptor antagonist and has a neuroprotective effect, while quinolinic acid acts as an NMDA receptor agonist and exhibits a neurotoxic effect[145]. In major depressive disorder and bipolar disorder, a decrease in tryptophan and kynurenine was noted. In these mental disorders, there is a shift in tryptophan metabolism from the serotonin pathway to the kynurenine pathway[146]. Tryptophan metabolites are involved in the pathogenesis of various neurodegenerative disorders (Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease, Parkinson's disease) as well as other diseases such as AIDS, cancer, cardiovascular disease, inflammation and irritable bowel syndrome[147,148];

- Participate in the regulation of activation, proliferation and migration of immune surveillance cells (T- and B-lymphocytes, macrophages and natural killer cells) and the production of inflammatory signaling molecules, cytokines, nitric oxides and superoxides[149,150];

- Influence the motility of the gastrointestinal tract. In particular, as a result of tryptamine action on the serotonin receptor 5-HT4R[151];

- Indoles may contribute to the development of cardiovascular, metabolic and psychiatric diseases[152].

Secondary bile acids

It is worth noting that secondary bile acids play a special role in the development of inflammation and colorectal cancer (CRC). It is known that between 5% and 10% of nonreabsorbed bile acids can undergo biotransformation into secondary bile acids as a result of bacterial metabolism involving bacterial bile acid hydrolases (BSHs). Most BSH bacteria are gram-positive enteric bacteria, including *Clostridium*, *Enterococcus*, *Bifidobacterium*, and *Lactobacilli*. The only gram-negative bacteria with BSH activity are members of the genus *Bacteroides*[85,153]. Interestingly, secondary bile acids at high concentrations associated with Western diets can induce oxidative/nitrosative stress, ROS production, DNA damage, apoptosis and mutations[85,154,155] and induce proinflammatory macrophage M1 polarization[154] by binding secondary bile acids with Takeda G protein-coupled receptor 5 (TGR5)[156], thereby initiating inflammation[157].

Several studies have noted that through the activation of the epidermal growth factor receptor (EGFR), secondary bile acids can induce COX-2 expression, stimulate EGFR-MARK signaling[155,158], activate cellular β-catenin signaling and the nuclear factor kappa B (NF-κB) pathway[155,159], and provide transfer of extracellular signal-regulated kinase 1 and 2 (ERK1/2) *via* activator protein 1 (AP1) and c-Myc, thereby stimulating the proliferation and invasiveness of colon cancer cells[85,160,161]. At the same time, at low and physiological concentrations, secondary bile acids can have an anti-inflammatory and antitumour effect through a reduction in proinflammatory cytokine levels[162,163]. In particular, an antitumour effect has been noted for lithocholic and ursodeoxycholic acid (UDCA), which are secondary bile acids produced by *Clostridium* species. Lithocholic acid (LCA)at concentrations corresponding to its tissue reference concentrations (< 1 μM) has an antitumour effect on breast cancer cells by inhibiting the epithelial-mesenchymal transition, reducing the production of vascular endothelial growth factor (VEGF), and activating antitumour immunity[164]. Ursodeoxycholic acid (UDCA) may prevent the development of CRC by regulating oxidative stress in colon cancer cells[165]. However, the preventive effect of UDCA on CRC is not universally accepted[166].

Trimethylamine N-oxide (TMAO)

TMAO is a molecule resulting from the oxidation in the liver of a microbial metabolism product, trimethylamine (TMA). TMA is formed in the colon from choline, betaine, and carnitine. The main food precursors of TMA are red meat, fish, poultry, and eggs. TMA from the colon is absorbed into the bloodstream, where it is oxidized by the hepatic enzyme flavin-containing monooxygenase-3 to TMAO, most of which is then excreted unchanged in the urine. Plasma TMAO levels are determined by several factors, including diet, age, gut microbiota, drug intake, and liver flavin monooxygenase activity. The main TMA producers are *Clostridia*, *Shigella*, *Proteus*, *Aerobacter*, and *Eubacterium sp.*[167,168]. Some bacterial enzymes are able to oxidize TMA to TMAO in the colon[169]. At the same time, TMAO can be metabolized into dimethylamine, formaldehyde, ammonia and methane by some methanogenic bacteria, which leads to its depletion in the colon[170]. It cannot be excluded that the production of formaldehyde

under oxidative stress conditions caused by TMAO metabolism may be one of the factors contributing to the induction of CRC. In the experiment, intragastric administration of a suspension of CaCO3 in a mixture with formaldehyde and hydrogen peroxide induced tumors of the stomach and cecum in rats[171].

It has now been established that elevated plasma levels of TMAO correlate with the risk of developing atherosclerosis[172-175], obesity[175,176], cardiovascular diseases[174,177,178], type 2 diabetes[175], chronic kidney disease[179,180] and CRC[181,182]. Elevated TMAO levels have been associated with endothelial dysfunction and inflammatory damage to the vascular endothelium[183,184], an increase in the level of proinflammatory cytokines, and a decrease in the level of anti-inflammatory cytokines[185,186] with the activation of the MAPK and NF-κB transcriptional pathways[185], oxidative stress[177], cell proliferation and angiogenesis[187]. Xu *et al* (2015) provided evidence that these risks may be genetically determined[188]. Moreover, it is worth noting that the effects of TMAO may differ between healthy and diseased conditions. In healthy individuals, TMAO can demonstrate protective, antioxidant or anti-inflammatory effects, while in patients, especially under conditions of oxidative stress, it can have a negative impact on human health[189]. Further research is needed to elucidate the effects of TMAO on human health.

Hydrogen sulfide

Hydrogen sulfide is a metabolite of sulfate-reducing bacteria that metabolize dietary sulfates and other sulfur-containing compounds, including taurine[155]. It is produced by a wide range of *Enterobacteria*, primarily of the genus *γ-Proteobacteria*. The hydrogen sulfide concentration in the large intestine is more than 5 times higher than in the small intestine[190]. Hydrogen sulfide, similarly to secondary bile acids and TMAO, can multidirectional affect inflammation, oxidative stress, and carcinogenesis[155,191]. Various authors have demonstrated both its inflammatory[192] and anti-inflammatory effects[193,194], as well as its carcinogenic[195,196] and anticancer properties[197,198]. For example, some researchers have demonstrated that hydrogen sulfide may be associated with the breakdown of disulfide bonds in the mucus double layer in the colon wall,

leading to inflammation and translocation of bacteria and toxins[199]. At the same time, other researchers suggest that hydrogen sulfide can protect the mucus layer and repair this already destroyed, thereby preventing inflammation[190]. It is believed that the physiological and pathological effects of hydrogen sulfide are associated with its concentration[200]. Given that intracellular hydrogen sulfide has a significant impact on many cellular functions, such as tight junctions, autophagy, apoptosis, vesicle trafficking, cell signaling, epigenetics and inflammasomes[201], and can be used as a therapeutic agent, its further study may open new opportunities in understanding the mechanisms of the development of pathological conditions and their treatment.

Polyamines

Polyamines are versatile polyfunctional molecules involved in cell proliferation and differentiation, apoptosis, angiogenesis, immune response, signaling, and gene expression[202,203]. They can be supplied with food and can form as a result of endogenous synthesis, as well as a result of bacterial metabolism, such as putrescine, spermidine and cadaverine[204,205]. In particular, cadaverine is formed from lysine by decarboxylation with the participation of lysine decarboxylase. Putrescine is formed by decarboxylation of ornithine catalyzed by ornithine decarboxylase. Spermidine synthase is involved in the formation of spermidine from putrescine. These enzymes are produced by most gram-negative bacteria[205,206]. Polyamines synthesized by the intestinal microbiota are then transported into the bloodstream through the colonic mucosa[207].

It has been established that polyamines produced by intestinal bacteria suppress chronic inflammation and strengthen the intestinal barrier in the colon, contribute to a significant improvement in the host's cognitive functions and increase life expectancy in experimental animals and have a cardioprotective effect[203,208,209]. In a number of studies, the antitumour effect of cadaverine was noted. In an experiment, cadaverine caused a decrease in the proliferative activity and invasiveness of breast cancer cells and contributed to the induction of mesenchymal-epithelial transition, a decrease in the stemness of cancer cells and their ability to metastasize[210]. In breast cancer, a decrease

in the intestinal biosynthesis of cadaverine was noted, especially in patients with carcinoma in situ and stage I of the disease. With a high expression of bacterial LDC in the gut contents, a significantly longer survival was noted than with a low expression[210].

At the same time, a number of studies have noted that high levels of polyamines may be associated with tumor transformation and cancer progression[211]. Thus, in CRC, an increase in the levels of bacterial cadaverine and putrescine in the feces was noted[212,213]. Huang *et al* (2020) reported that increased spermine intake is associated with an increased risk of CRC, while a higher intake of total polyamine, putrescine and spermidine is significantly associated with a reduced risk of CRC[214]. It is believed that the procarcinogenic effect of polyamines is associated with the activation of the PTEN-PI3K-mTOR (TORC1), WNT, and RAS pathways[211].

Microbiome and biotransformation of xenobiotics

The gut microbiome can influence the biotransformation of a number of xenobiotics with known carcinogenic properties, such as heterocyclic amines (HCAs). HCAs are formed during thermal processing (frying, baking, grilling, *etc.*) of various food products, including oils, grains and vegetables, but especially processed meat[215]. HCAs have pronounced genotoxic and mutagenic properties, contributing to the development of malignant neoplasms of the intestine, liver, lungs, breast and other tumors. The carcinogenicity of HCAs is associated with mutations in proto-oncogenes and tumor suppressor genes, including K-ras, Haras, Apc, β-catenin and TP53[216]. The intestinal microbiota can metabolize HCAs into molecules with increased mutagenic activity[217]. At the same time, the intestinal microbiota can bind or metabolize food-derived HCAs, facilitating their excretion with feces or conversion into less toxic compounds[215,218]. These processes involve bacterial beta-glucuronidase (B-GUS) and glycerol/diol dehydratase (GDH) produced by some lactic acid bacteria and probiotics. A decrease in the number of taxa with B-GUS and GDH activity was noted in patients with CRC[219].

DYSBIOSIS AND HUMAN DISEASES

According to the results of numerous studies, in many diseases, including inflammatory bowel diseases[113,220-224], chronic liver diseases[225-227], obesity[228,229], diabetes mellitus[230,231], osteoporosis[41,232], cardiovascular[233,234] and oncological diseases[212,213,235], a decrease in the microbiota diversity and an overgrowth of pathogenic and conditionally pathogenic flora are observed[50,236,237]. Interestingly, in CRC, Fusobacterium nucleatum was found not only in the primary tumor but also in metastatic lesions in the liver[238] and lung[239]. In addition, it has been noted that dysbiosis to a certain extent can influence the development of depression, bipolar depression and schizophrenia[240], as well as autism[241] and Parkinson's disease[242,243]. Figure 1 demonstrates the association of gut dysbiosis with various human diseases.

It should be emphasized that the diseases associated with dysbacteriosis are multifactorial in nature[50,236,237,244] and are associated with bacterial invasion through the physical and chemical barriers of the gastrointestinal tract[50]. It is hypothesized that diet and other environmental influences may change the microbiome and thus provoke an unstable basis of genetic predisposition, which may lead to disease development, at least in some patients. Dysbiosis may be related to heredity, use of antibiotics, proton pump inhibitors, certain types of chemotherapy, advanced age, diet, and other factors[245,246]. At the same time, it should be noted that numerous studies have not revealed typical changes in the microbiome for a particular pathology[247]. A detailed analysis of host metabolism (metabolic index) and habitual diet (including the consumption of plant and animal foods, fermented milk probiotics, such as yogurt) allowed Asnicar *et al* (2021) to establish consistent gut microbiome signatures, segregating favourable and unfavourable taxa with multiple measures of both dietary intake and cardiometabolic health[248]. However, we believe the issue cannot be considered definitively resolved, since it is not completely clear what is primary: a violation of the microbiome that then contributes to the development of diseases or disorder can cause changes in the microbiome. These questions require further research.

MODERN APPROACHES TO THE REGULATION OF THE GUT MICROBIOME

Considering that intestinal dysbiosis plays an important role in the development of a number of diseases, normalizing the microbiome seems to be a promising direction for their treatment. There are several approaches to solve this problem.

"**Mediterranean diet**". It is well known that a Mediterranean diet is rich in vegetables, fruits, whole grains and fish and thus creates favourable conditions for beneficial bacteria such as *Firmicutes*, which are involved in the production of butyrate necessary to maintain a healthy barrier between the colon and blood flow, preventing inflammation in the gut[64]. Unlike the Mediterranean diet, a "Western" high-fat, low-fiber diet contributes to colon inflammation and cancer[249,250].

Prebiotics are substrates that are selectively utilized by host microorganisms to provide health benefits[251]. The use of prebiotics, such as dietary fiber, reduces obesity and has anti-inflammatory and anticancer effects[85,250,252,253]. Dietary fiber intake is associated with a lower incidence of colon cancer, since fiber reduces the concentration of intestinal carcinogens due to increased stool mass, intestinal motility and production of butyrate, which maintain colonocyte health, increase apoptosis and inhibit cancer cell proliferation. A long-term fiber-rich diet increases the density of *Firmicutes*, which have the ability to mediate an immunomodulatory and anti-inflammatory immune response[64]. Moreover, dietary fiber physically interferes with fatty acid reabsorption and cholesterol absorption, thereby reducing the risk of obesity, diabetes, and atherosclerosis[254,255];

Transplantation of fecal microbiota (FMT). The mechanism of FMT is to restore the fermentation activity, pH and redox potential of the microbiome habitat in the respective niches and restore normal gas production and synthesis of SCFA[245,256]. FMT has been used in the treatment of nosocomial diarrhea caused by *Clostridioides difficile*[245] and other intestinal diseases[257-259]. Scibelli *et al* (2021) demonstrated the effectiveness of using FMT in the treatment of rectal fistula in a patient with a colostomy who received intensive antibiotic therapy for a long time due to trauma[260]. Impressive results have also been obtained in the treatment of Crohn's disease by FMT: nearly 60% of patients achieved a clinical response to treatment, and more than 20% of

patients experienced sustained clinical remission, including 2 of 6 patients with perianal fistula[261]. The use of FMT has been shown to be beneficial in hepatic encephalitis, metabolic diseases, neuropsychiatric disorders, autoimmune diseases, allergic disorders, tumors, Parkinson's disease, multiple sclerosis, myoclonus dystonia, chronic fatigue syndrome, and idiopathic thrombocytopenic purpura[257,262-264].

The disadvantages of FMT are frequent side effects such as constipation, diarrhea, bloating and possible transmission of potential pathogens[245,265]. Given the enormous promise of microbiota transplantation, the search for new methods and ways to use it continues[266]. Attempts have been made to replenish only bacteria that had certain characteristics and whose number was reduced[245,267,268], but reproducibility and standardization of preparations used for transplantation have been proven to be a problem[267]. Zhang *et al* (2020) first revealed that washed microbiota transplantation is safer, more precise and more quality-controllable than crude FMT by manual[269]. Currently, there are a series of clinical trials conducted for SER-109, which is a consortium from several species of *Firmicutes* isolated from the stool of healthy human donors and encapsulated. The use of the medication reduced the risk of recurrence of nosocomial diarrhea caused by *Clostridioides difficile* from 41.3% in the placebo group to 11.1% in the treatment group[270].

Probiotics are live strains of carefully selected microorganisms that, when administered in adequate amounts, confer a health benefit on the host[271]. Probiotics can be easily and economically prepared and given to patients daily in the form of yogurt, drinks, cheese or capsules[272]. They contribute to the maintenance of intestinal barrier function, have immunomodulatory, metabolic and antiproliferative effects[273,274], and regulate DC maturation by producing tolerogenic DCs (tolDCs), which can reduce inflammation[275] and synthesize antimicrobial substances[276]. The ability of *Bifidobacterium dentium* and its secreted factors to suppress endoplasmic reticulum (ER) stress genes and promote MUC2 secretion, as well as to secrete the antioxidant γ-glutamylcysteine, which reduces the formation of ROS and suppresses NF-kB activation and IL-8 secretion, has been established[277].

Currently, *Lactobacillus*, *Bifidobacterium* and *Saccharomyces* are used to treat hospital-acquired diarrhea caused by *Clostridioides difficile*[278]. Oral use of *Lactobacillus reuteri 6475* reduced the loss of total bone mineral density (BMD) in women aged 75 to 80 years with low BMD[279]. Dietary supplementation with soluble corn fiber at doses of 10–20 g per day has also been associated with an increase in calcium absorption and a larger number of *Clostridium* and unclassified *Clostridiaceae* in feces[280]. These data suggest that both probiotics (live bacteria) and dietary supplements needed to feed the bacteria can be used as therapeutic agents to combat osteoporosis. It is possible that the use of their combination will be even more effective.

For therapeutic purposes, commensals (bacterial strains that are resistant to certain types of pathogenic bacteria), bacteriophages and fungi can be used. For example, CBM588 is a probiotic composed of the commensal *Clostridium butyricum*, which produces large amounts of butyrate and activates neutrophils and Th1 and Th17 cells[281]. Bacteriophages are viruses that can infect and multiply in pathogenic bacteria and eventually lyse them[245,282]. Yeasts such as *Saccharomyces boulardii* and *C. albicans*, as well as fungal wall components such as β-glucans, can inhibit the growth of some enteric pathogens. *Saccharomyces boulardii* produces proteases or phosphatases that inactivate disease-causing toxins produced by gut bacteria and modulate multiple signaling pathways to suppress toxin-induced inflammation[245,283,284]. Because of the risk of fungemia, they should be used cautiously in debilitated patients[285].

CONCLUSION

The study of the influence of the gut microbiome on health and disease is one of the most relevant and interesting areas in modern science. The number of microorganisms inhabiting the human body is enormous, and their composition can vary significantly between individuals[248,286]. Dysbiosis has been found to play an important role in the development of a number of diseases, however, numerous studies have not revealed typical changes in the microbiome characteristic for a particular pathology[247]. It is likely that the impact of the microbiome and its metabolites on human health cannot be

considered only in terms of health benefits or harms. Metabolites that perform important functions in the human body under certain conditions can have a negative impact on human health, while metabolites that are considered potentially dangerous can be beneficial[287].

As already noted, changes in the quantitative and qualitative microbiota composition can lead to an increase in the production of potentially toxic metabolites, such as secondary bile acids, TMAO and hydrogen sulfide, and an increase in the risk of developing intestinal, cardiovascular, neurological, oncological and other diseases. These changes may be related to diet, lifestyle, age, medications, and other factors. Thus, taking antibacterial drugs can increase sensitivity to viral infections[288], increase the risk of developing malignant neoplasms[289], and contribute to the development of resistance to chemotherapy drugs and immune checkpoint inhibitors in cancer patients[290]. Interestingly, some authors attribute an increase in the risk of malignant neoplasms with the use of antibiotics to a decrease in the synthesis of intestinal metabolites with antitumour activity, for example, lithocholic acid and cadaverine[289]. However, in some malignant neoplasms, the antitumour effect of antibacterial drugs was demonstrated. For example, in CRC, antibiotic therapy had a cytostatic effect due to the destruction of bacterial biofilms, the formation of which was associated with polyamines[291]. In an experiment, adding broad-spectrum antibiotics to drinking water for 3–4 wk reduced age-related oxidative stress and arterial dysfunction in mice[292]. It is believed that microbiota modulation using antibiotics, probiotics, fecal microbiota transplantation or nanotechnology can be effective in a variety of diseases, including enhancing the antitumour effect of chemotherapy drugs or immune checkpoint inhibitors[290].

It should be noted that despite the huge amount of research on the role of the microbiome in health and disease development, there are a number of issues that deserve further study. In particular, it is known that the microbiota affects the mood and behavior of a person, his physical activity, resistance to stress and diseases. At the same time, whether mental and physical health influence the human microbiome is not

well understood. Many questions remain regarding the role of the gut microbiome in drug metabolism, the development of drug resistance and chemoresistance, and the role of the microbiome in cancer progression. Answers to these and other questions are still waiting for their researchers.
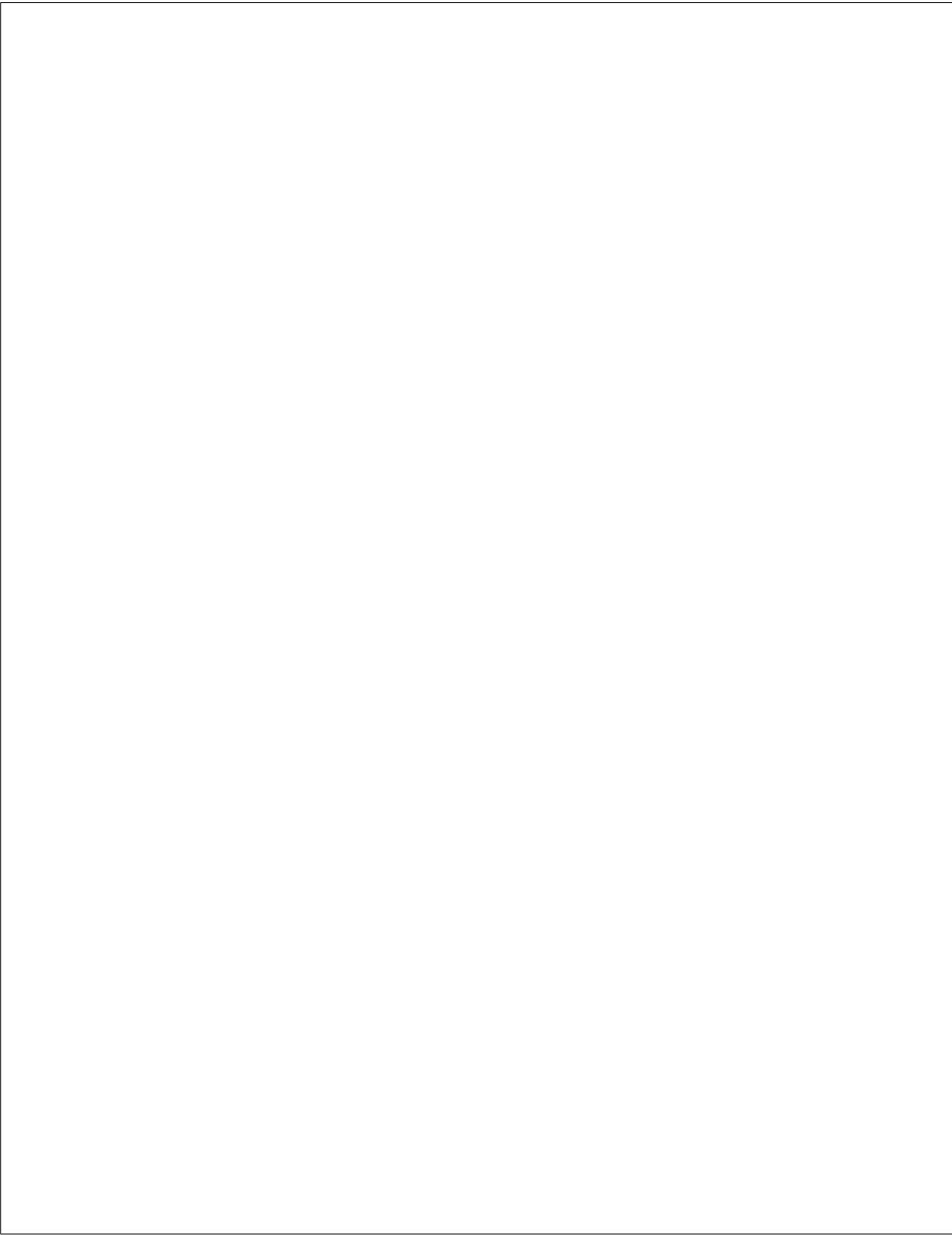
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