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Effect of Bacteriophages in the Human Body

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ABSTRACT

Recent data demonstrating the presence of phages in the human body open new and exciting perspectives for research on the actual significance of such body phageome. Bacteriophages (phages) are bacterial viruses that are detected in soil, water and in the human body (feces, saliva, sputum, blood, and urine). Phages can be found from blood and other tissues after administration through different routes. Interestingly, phages may pass the intestinal barrier and get translocated to blood, lymph and internal organs currently, while the protective effects of such phages have been initially suggested, the recent reports suggest potential pathogenic effects of such phages. Moreover, there is also ample evidence indicating that phages do not adversely affect the immune system and do not cause any harmful effects, even those present in cerebrospinal fluid. It has been pointed out that the effects of phages on the immune system may be phage-specific, which is probably most close to the truth. Further studies are necessary to shed more light on those phenomena, which would determine the multifaceted role of endogenous phages and their significance for further progress of phage therapy

Keywords: Phages, Intestine, Human body, Urine, Virus

INTRODUCTION

Bacteriophages (phages) are bacterial viruses that are detected in soil, water and in the human body (feces, saliva, sputum, blood, and urine) [1]. Phages can be found from blood and other tissues after administration through different routes. Interestingly, phages may pass the intestinal barrier and get translocated to blood, lymph and internal organs. Intestinal epithelial cells (IECs) play an essential role in the absorption of nutrients and constitute a barrier against potentially harmful microbial antigens [2]. The in vivo role of epithelial cells in the uptake of lumen antigens such as viruses, bacterial cell debris and dietary particles was investigated in mouse experiments. Goblet cell-associated passageway (GAP) enables the entry of soluble protein antigens, but not inert particles (0.02–2 μm) into lamina propria (LP), the tissue that lies under the epithelium [3]. Small molecular weight antigens such as chicken ovalbumin, dextran, and bacterial LPS enter the LP via GAP [6]. However, epithelial cells overlying the villi can internalize antigens such as bacterial cell debris and inert nanoparticles, which are then found colocalizing with the CD11c+ dendritic cells in the LP. 20–40 nm nanoparticles are taken up readily by IECs, while nanoparticles larger than 100 nm are taken up mainly by the epithelial cells overlying Peyer's patches [2].

The intestines are predominantly inhabited by phages and, to a lesser degree, eukaryotic viruses. One could expect as estimated that 10^{15} phages reside in the human gut, which accounts for approximately 10^{8}–10^{10} phages per gram of human stool depending on the extraction method used [4] and ~10^{9} bacterial cells per gram of stool. As estimated by month one of infants life virus-like particles (VLPs) counts averaged 1.6×10^{9} per gram of stool, at month 4 were similar [5]. In 2-to 5-year-old children, VLPs counts averaged 9.4×10^{8} per gram of stool. This is similar to that estimated for adults. For months one and four, most samples were positive for bacterial 16SrRNA gene sequences with a median value of 3.1×10^{8} [5]. Individual specificity and temporal stability of the human gut virome were observed for up to 1 year. Although, phage content in human stool samples may vary depending on the health status of the donor in some gastrointestinal disorders. Phages can potentially alter gut bacterial microbiota. It has been suggested that changes in phage composition may play a role in some diseases. Phage may

also play an important role in microbiota homeostasis. They may regulate microbiota diversity and may be involved in an increase in gut permeability and immune disturbances [6].

Phages in Healthy Individuals

Intestinal microbiota consists of bacteria, viruses (including phages) and fungi. The small intestine contains such bacteria as *E. coli*, *Streptococcus* spp. and *Bacteroidetes*. The large intestine and stool samples include the dominant bacteria *Firmicutes* and *Bacteroidetes* [7]. Phages that are detected in the intestine belong to the order *Caudovirales* with double-stranded DNA (families *Siphoviridae*, *Myoviridae* and *Podoviridae*) as well as the other phages with single-stranded DNA (families *Microviridae* and *Inoviridae*) [7]. The prevalence of crAssphage infecting *Bacteroidetes* in fecal samples in half of the healthy individuals from 4 cities in two continents was observed [8]. Most phages in different places in the human body are temperate. The fecal samples from healthy individuals contained mainly temperate phages (56.5%) vs. virulent phages (2.5%), whereas patients with digestive or respiratory system diseases and leukemic disease also had temperate phages (54.8–56%) vs. higher frequency of virulent phages (13–20%) compared with healthy individuals [9]. Lower titers of temperate phages were found in fecal samples collected from healthy individuals and from patients, whereas higher titers of virulent phages were detected from patients. The temperate phages belonged mainly to λ type and φ80 type [9]. A study of HLA-matched infants up to 3 years of age was performed. In 10 cases with autoantibodies (6 with no progression to type 1 diabetes (T1D) and 4 who developed (T1D) and 8 cases non-seroconverted control individuals), most intestinal E. coli phages were temperate (63 phages) and only in a few samples were lytic Enterobacteria phages found. However, a recent study indicated that the gut virome of 9 out of 10 healthy human subjects had no dominance of phage sequences harboring marker genes of temperate phages (integrase and site-specific recombinase genes) [10]. These results led the authors to propose that mechanisms other than the integration of the phage genome into the host may be responsible for the long-term persistence of phages in the human gut. Sutton and Hill (2019) discussed actual data regarding the human virome and the influence of phages on gut microbiome shape [11].

According to current knowledge, phages are the most predominant element of the human microbiome, and they are believed to play a crucial role in shaping microbial composition driving bacterial diversity and facilitating horizontal gene transfer. Double-stranded DNA phages of the order Caudovirales are one of the dominant fractions of the gut virome. The concept of a core human virome was based on the fact that 23 core phages (based on the presence of phage contigs from the healthy individuals) were observed in more than 50% of samples from an independent group of 62 healthy individuals. In a differing opinion, the human gut microbiome is highly individual-specific at a sequence level [11]. Additionally, it was reported that phages that colonize the gut are unique to each individual and with a high degree of interpersonal variation [27]. Intestinal mucosa plays an essential role in maintaining human health. The quantitative and qualitative differences are between phages of healthy individuals and patients [12]. Zuo et al. (2019) indicated a lower abundance of Caudovirales phages in the gut mucosa of healthy individuals, but a higher richness and diversity in these phages were observed in comparison to ulcerative colitis (UC) patients [13]. Manrique et al. (2017) suggest that an adequate balance between lysis and lysogeny in the human gut is essential to maintain human health [14]. In a healthy human gut, only a small part of the prophage reservoir is activated and found as extracellular phages. The model of the shift from health to disease has been proposed, which was based on the increase of some prophage induction, which is likely sensitive to stress. Therefore, the process, such as inflammation in patients with inflammatory bowel disease (IBD), may lead to an increase in prophage activity [14]. The increase in lytic phages in stool has also been correlated with the digestive system and leukemic diseases [4]. Another study emphasizes that phage particles may harbor antibiotic resistance genes (ARGs) in healthy individuals [15]. One hundred fifty fecal samples from healthy individuals who did not receive antibiotic treatment in the 3 months prior to sample collection were examined. In 72.7% of stools of healthy individuals, at least one ARG in phage DNA was found.

Phages in Diseases

Reports have demonstrated that human gut microbiota may be altered in various disorders such as obesity, diabetes, metabolic disorders, diarrhea, IBD, and malnutrition [16]. Garmaeva et al. (2019) reported that it is difficult to determine whether changes in the virome and microbiome are a cause or an effect of the disease. Koch's criteria refer to the causal relationship between microbe and disease. The metagenomic version of Koch's postulates relates to significant differences in metagenomic traits between sick and healthy individuals and evidence that inoculation of samples from sick to healthy animals leads to the induction of the disease. Only some criteria are supported in human phage research, e.g., significant differences in viral contigs or specific phages between sick and healthy individuals, and only a few animal studies have been performed and mostly with fecal microbiota transplantation (FMT) application rather than inoculation of phages [18].

Recent studies have indicated an increase of gut permeability in mammals (a rat model) after exposure to commercial phage cocktails (lysate) against Enterobacteriaceae, Staphylococcaceae, Streptococcaceae and Pseudomonadaceae developed by Microgen (Moscow, Russia) (1×106 pfu/mL) and administered orally for 10

days [19]. The authors showed that phage lysates could cause inflammatory reactions by, among others, increasing the level of circulating endotoxin as a result of changes in the intestinal microflora and an increase in intestinal permeability. In their research, they showed differentiation in the composition of the microbiome before and 10 days after administration of the phage cocktail. It has been shown to reduce the number of bacteria from Actinobacteria, Deferribacteres, and Proteobacteria and to reduce bacteria from Spirochetes, Tenericutes and TM7. It is well known that phage administration reduces the number of Lactobacillus and Faecalibacterium species that are very beneficial to mammalian organisms. The phages used in the studies did not directly affect both of these species, but other phages may lead to microbial changes, which may affect the macroorganism [19].

The virome of the human gut has been studied in a number of diseases with a connection to changes in the composition of known phages from Caudovirales, but till now, little is known about any role of the virome in disease [11]. The latest report reveals a highly interactive and dynamic community in the gut microbiome where lytic phages coexist and knockdown targeted bacteria and ultimately modulate the gut microbiome. Moreover, the authors showed that phages have the ability to modulate the bacterial community with influence on the production of metabolites, such as neurotransmitters, amino acids, and bile salts, which are known to affect the mammalian host. Norman et al. (2015) showed significant associations between the expansion of the Caudovirales phages and specific members of the bacterial community in the IBD disorder [20]. The authors showed that in Crohn's disease (CD) and ulcerative colitis (UC) patients, the enteric virome demonstrated an abnormal form, and they are disease- and cohort-specific. It did not appear that expansion and diversification of the enteric virome were secondary to changes in bacterial populations. It is believed that changes in the virome may contribute to intestinal inflammation and bacterial dysbiosis and that they take part in inflammatory bowel disease. As the main part of the human virome, the Caudovirales phages may play an essential role in the physiology of the intestine and the bacterial microbiome composition. The geographic variation of phages and specific bacteria, as well as unique changes of phages in CD and UC patients (each disease harbored unique phages), were found. An increase in differences in richness and certain taxa of Caudovirales phages between CD and UC was observed by the authors. In the CD and UC patients, a significant reduction in bacteria diversity and bacterial richness, as well as the specific virome for CD patients compared to household control, was found. As a consequence of bacterial lysis, via proteins, lipids, nucleic acids pathogen-associated molecular pattern (PAMPs, antigen release and direct interactions between phages and the mammalian host, the chronic intestinal inflammation leading to the destruction of intestinal tissue in IBD pathology have been observed [20].

It additionally has been postulated [21] that phages have unique binding sites to inflammatory mediators. T4 phages revealed that the needle domains contain seven iron ions coordinated by histidine residues. The tail adhesin gp12 mediates adsorption of T4 phages to E. coli cells, whereas recombinant gp12 binds to LPS and also prevents LPS-induced production of proinflammatory cytokines in mice [22]. It is believed that phages can cross the epithelial cell layer and interact with underlying immune cells. Lehti et al. (2017) described that phages could be internalized by eukaryotic cells by binding to moieties that resemble bacterial phage receptors. The presence of an integrin-binding motif in genetically engineered filamentous phage was shown by Namdee et al. (2018) [23]. Nguyen et al. (2017) showed phage internalization by endocytosis and that they were transferred through the Golgi apparatus [24]. The immunoregulatory effect of T4 phages manifested as a reduction in reactive oxygen species (ROS) production by peripheral blood polymorphonuclear leukocytes stimulated by LPS was demonstrated by Międzybrodzki et al. (2008) [25]. Moreover, it has been shown that NF-kB activity may be modulated by the Staphylococcus aureus phage JS25 by inhibition of the production of several proinflammatory cytokines and inhibited NF-kB signaling [26]. Zimecki et al. (2003) demonstrated that the S. aureus A20/R phage mediates the costimulatory activity of splenocyte proliferation and proinflammatory cytokine IL-6 production. An in vitro study with S. aureus and Pseudomonas aeruginosa phages demonstrated production of both pro- and anti-inflammatory cytokines from peripheral blood mononuclear cells following endocytosis of purified phage virions (the prevailing effect being anti-inflammatory) [17]. Additionally, these results are supported by the proposed ability of phage virions to cross the mammalian epithelial barrier in vitro via peptide sequences expressed on the phage capsid surface [3]. Furthermore, the E. coli PK1A2 phage can interact with polysialic acid at the surface of the eukaryotic cell lines, which has a structural similarity with polysialic acid polysaccharide of the bacterial host.

The E. coli PK1A2 phage from the Podoviridae family within the supergroup T7, subgroup SP6 is a natural variant of the PK1A phage, which was isolated because of its ability to bind bacteria containing the K1 polysialic acid capsule with a structure identical to the polysialic acid present on mammalian cells. The PK1A2 phage is closely related to the E. coli K-1 specific phage K1E and propagated on E. coli host strain IH 954 with a highly reduced amount of K1 capsule. The authors showed in in vitro studies that the phages bind to polysialic acid and that they progressively internalize to the live eukaryotic neuroblastoma cell line SK-N-SH, SK-N-AS, human foreskin fibroblast cell line BHK-21 from the American Type Culture Collection (ATTC) [18].

Impact of Phage Intake on Gastrointestinal Human Health

Febvre et al. (2019) revealed that supplemental phage intake had no significant impact on gut microbiota and overall health status [21]. In a double-blinded placebo-controlled study, they applied a phage cocktail E. coli for 28 days to healthy individuals. They determined the effect of a phage cocktail on gut microbiota and markers of intestinal and systemic inflammation. The applied phage did not globally disrupt the microbiota. However, during the study, specific bacteria were altered; that is, there was an increase in the members of Eubacterium and a decrease in the taxa related to Clostridium perfringens. Moreover, inflammatory markers and lipid metabolism were unaltered. A small decrease in circulating IL-4 was observed [20].

In a small intestinal in vitro model, researchers showed similar results [22] as Febvre et al. [20] of using strain-specific coliphages to target E. coli. The phage cocktail E. coli was similar to ciprofloxacin in reducing E. coli by 2–3 log but had a much milder influence on commensal non-targeted bacteria compared to the antibiotic [22]. Considering that only E. coli phages were applied in both studies [22], one could question whether it really is surprising that no significant global phenotypic effects are observed since the commensal E. coli only represents a minor fraction of the normal gut microbiota. Both Hsu et al. (2019) and Fazzino et al. (2020) have shown how other than coliphages indirectly may affect other members of the gut microbiota. Hsu et al. (2019) indicated in a mouse model that lytic phages not only knockdown their bacterial hosts but also affect non-susceptible commensal bacteria in the gut through cascading effects. Fazzino et al. (2019) demonstrated in laboratory experiments that the attack of Salmonella enterica with specific P22 vir phage delayed community growth with little effect on final species ratios. On the other hand, the E. coli-specific T7 phage attack altered the final species ratios in favor of S. enterica and caused a small delay of community growth.

The PHAGE study, a double-blinded placebo-controlled study, defined the safety and tolerability of phages in healthy adults with mild to moderate gastrointestinal distress [24]. Clinic visits of participants took place at the Colorado State University Human Performance Clinical Research Laboratory before and after each 28-day treatment. Participants used an E. coli phage cocktail or placebo (1 capsule daily) for 28 days, followed by a 2-week washout period and 28 days of the opposing treatment. The study showed the safety and tolerance of phages in the human population being studied and even some anti-allergic effects (lowering of cytokine IL-4 serum levels) [25]. The study suggests that phages may be applied as a dietary supplement in healthy individuals with mild to moderate gastrointestinal distress without causing exacerbation of symptoms. Future analyses may explain the effect of phage consumption on the gut microbiota and intestinal and systemic inflammatory markers [25].

CONCLUSION

Recent data demonstrating the presence of phages in the human body open new and exciting perspectives for research on the actual significance of such "body phageome". Currently, while the protective effects of such phages have been initially suggested, the recent reports mentioned above also suggest potential pathogenic effects of such phages. Moreover, there is also ample evidence indicating that phages do not adversely affect the immune system and do not cause any harmful effects, even those present in cerebrospinal fluid. It has been pointed out that the effects of phages on the immune system may be phage-specific, which is probably most close to the truth. Further studies are necessary to shed more light on those phenomena, which would determine the multifaceted role of endogenous phages and their significance for further progress of phage therapy.

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