Mini Review
Volume 5:1,2020
DOI: 10.37421/jib.2020.5.145

Journal of Immunobiology

ISSN: 2476-1966 **Open Access**

Bacteriophage at the Gastrointestinal Mucosal Barrier and Regulation of Mucosal Immunity

Amanda Carroll-Portillo[1] and Henry C Lin[1,2*]

[1]*Division of Gastroenterology and Hepatology, University of New Mexico, Albuquerque, NM 87131, USA*

[2]*Medicine Service, New Mexico VA Health Care System, Albuquerque, NM 87108, USA*

Abstract

The gastrointestinal system offers a unique opportunity for observing the interactions between commensals of the microbiome and the host immune system that is functioning close to these micro-organisms. Bacteriophages are critical to maintenance of homeostasis in this environment, and influence immune responses both indirectly, through modulation of the bacterial population and directly, through specific interactions with the metazoan host. Understanding these interactions in health and in human diseases is critical for developing novel treatment. This focused review summarizes the current knowledge in this area.

Keywords:

Bacteriophages • Homeostasis • Gastrointestinal microbiome • Pathogens • Phagocytosis

Introduction

Bacteriophages (phage), viruses that prey on bacteria, are a major component of the gastrointestinal microbiome. Phage replication, simplified, consists of a lytic cycle-propagation results in bacterial lysis- or a lysogenic cycle-phage incorporate their genome into the bacterial host [1,2]. In reality, life cycles are more complex [3] with phages that are able to undergo lysogeny (temperate) and phages that are not (non-temperate). Phage life cycle can greatly influence a bacterial population with shifting to different phases possibly associated with disease [4]. Phages are also thought to be specific to their bacterial host, but more recent data suggest some phages may bind bacteria more promiscuously [5,6] thus expanding the ability of individual phage to regulate a variety of bacteria. The interactions that phages have with bacteria in their environment are important for establishing which phages and bacteria are the dominant community members and have important downstream effects on metabolism and overall community structure.

Within the gastrointestinal microbiome, both bacteria and phage play a fundamental role in the development and maintenance of the metazoan immune system. From birth, they colonize the gastrointestinal tract, but the number and diversity of phages outnumber the bacteria [7,8]. A reversal of this dynamic comes with age; phage number and diversity lessens and stabilizes while bacterial numbers and diversity increase. Commensals are of importance to a healthy immune system as they are required for the development of IgA producing plasma cells, CD4+ T-cells, isolated lymphoid follicles, and invariant natural killer T cells [9-12]. Once established, the microbiome continues to be critical for maintenance of a healthy gastrointestinal system. Bacterial members provide protection from invading pathogens by physically blocking access and secreting soluble factors inhibitory to colonization of pathogens [13,14]. They process indigestible material and provide metabolites for the health of the system [15,16], and they can aid in regulating inflammation [17,18]. Because phage influences the overall bacterial community structure and composition, they too impact the metazoan host in ways that are just beginning to be appreciated. In addition to their indirect effects (via bacteria), phages can directly interact with Eukaryotic cells of the gastrointestinal system to mediate immune responses. In this review, we focus solely on phages at the gastrointestinal epithelium and their relation to immunity.

Phage and Healthy Gastrointestinal Epithelium

Within a healthy gastrointestinal system, the ratio of free phage to bacteria is 0.1 to 1:1, lower than that in other environments where phages reside, such as the ocean [4], suggesting a prevalence of temperate phages in lysogeny [2,19,20]. The distribution of phages in the intestine is hypothesized to occur as a gradient with lysogeny occurring in the lumen and outer reaches of the mucin layer, and lysis dominating its deeper parts [21]. Bacteria from the phyla Firmicutes and Bacteroidetes make up about 90% of the bacterial component of the microbiome [22]. Members of the Firmicutes and Proteobacteria phyla, also found in the microbiome, harbor the most lysogenic prophage as compared to members of the Bacteroidetes and Actinobacteria phyla [23,24]. Lysogeny at such high levels allows for

***Corresponding author:** Henry C Lin, MD, Medicine Service, 1501 San Pedro SE, New Mexico VA Health Care System, Albuquerque, NM 87108; Email: helin@salud.unm.edu*

***Received:** June 22, 2020; **Accepted:** July 08, 2020; **Published:** July 15, 2020*

phages to take advantage of a thriving bacterial environment while also allowing for a hidden "stock" of phages that are releasable upon recognition of particular bacterial signals. Presence of only phages utilizing lysis within the mucin may represent the advantages of these phages in binding mucins or other structural proteins. For example, Barr et al. identified a protein in the capsid of T4, a coliphage, that binds specifically to mucin [25,26]. This adaptation is beneficial to T4 as it allows for its retention in the mucin despite sloughing, and allows phage to move using the mucin web so as to increase the likelihood of encountering its bacterial prey.

Indirect and direct immune regulation

Indirect phage effects on the immune system are mediated by bacteria via phage-bacterial interactions. Commensal bacteria protect against pathogens through physical and soluble inhibition, and phages affect this protection by regulating bacterial abundance and diversity. Phage-driven lysis releases bacterial epitopes to be recognized by PAMP (pathogen-associated molecular pattern) receptors on eukaryotic cells, initiating immune signaling [27]. Both temperate and non-temperate phages influence bacterial metabolism resulting in downstream effects on cellular signaling that relies on bacterial metabolites [28].

Phages may also interact with the immune system more directly. Environmental sampling by immune cells could capture phage for processing. Phagocytosis of phage by macrophages and dendritic cells has been demonstrated. Once phages are intracellular, they are processed through lysosomal pathways for antigen presentation [29,30]. Entry of phage into eukaryotic cells is not solely an outcome of phagocytosis. In healthy subjects, phages have been found throughout the body displaying their success in overcoming the epithelial barrier of the gut. Because the tight, intercellular junction between epithelial cells is limited to molecules under 0.4 nm [31], it is likely that phage undergo transcytosis. Indeed, Nguyen et al. has demonstrated the apical to basolateral movement of phages across several epithelial barriers in an *in vitro* system [32]. Crossing the epithelial barrier via transcytosis allows phages to come into direct contact with immune cells in the submucosal layer, potentially stimulating immune signaling through a variety of mechanisms [33].

Phage and the Dysbiotic Gastrointestinal Epithelium

Loss of homeostatic balance among the gut microbial community, or dysbiosis, occurs in several gastrointestinal diseases including ulcerative colitis, Crohn's disease, and small intestinal bowel overgrowth. There are multiple physiological changes associated with dysbiosis including substantial changes in mucin secretion, inflammation, loss of barrier integrity, and increased infiltration of immune cells. Shifts in the gastrointestinal microbiota during dysbiosis result in loss of diversity as well as decreases in beneficial bacteria from the phyla Firmicutes. Concurrently, bacterial blooms of minor community members or establishment of bacterial pathogens are associated with increased epithelial inflammation [34,35]. While the role of phages during dysbiosis is not well understood, their communities also shift with, most frequently, an increased abundance of the tailed phages, order Caudovirales. The loss of Firmicutes bacteria, the main source of lysogeny, may stimulate switching among their temperate phages from lysogeny to lysis. This would be consistent with results seen in patients with irritable bowel disease (IBD) where the commensal *Faecalibacterium prosnitizii* is lost from the microbiome, but phages of these bacteria-found as prophage in healthy systems-are increasingly extracellular [36,37]. As some dysbiotic conditions are associated with a decrease or loss of mucin secretion, it is also possible this allows for phages typically embedded in the mucin to venture further toward the lumen and effect the bacterial populations they normally would not have access to.

Indirect and direct immune regulation

There are a number of mechanisms by which phages may influence the gastrointestinal immune system during dysbiosis. Similar to a healthy system, phages have great influence over the bacterial population in dysbiosis thereby indirectly influencing immune responses. If temperate phages become more lytic in the lumen [4] or if loss of mucin releases previously contained phage population into the lumen increasing predation, increased lysis of luminal bacteria would saturate the system with proinflammatory microbial antigens. Increased bacterial lysis would also affect luminal metabolic activity such that certain essential nutrients were no longer being produced for the epithelium to use. For example, with *F. prausnitzii*, which produces the short chain fatty acid butyrate as a metabolite. Butyrate helps maintain intestinal homeostasis and has a beneficial immunomodulatory effect [38]. Thus, if *F. prausnitzii* suffers from phage over predation and is lost in IBD, butyrate, a fuel for colonocytes, is also depleted leaving the intestinal epithelium in a weakened state.

Breakdown of the integrity of the gastrointestinal epithelial barrier accompanying inflammation provides ample opportunity for direct interaction between the immune system and bacteriophage. With phages able to recognize a variety of bacterial epitopes for binding, there is likelihood that molecular mimics exist on Eukaryotic cells that phages can also bind. Porayath et al. demonstrated that phage was able to bind to heparin and fibronectin, both found in the gastrointestinal epithelium [39], Lehti et al. showed that phage bound to the sialic residues on the surface of neuronal cells [40], and Shan et al. found *C. difficile* phages adhered to epithelial cells [41]. Additionally, with loss of integrity, the epithelium becomes much more permeable to larger molecules, including phages, offering an alternative to transcytosis for phages to move across the barrier. The combination of increased barrier traversion and influx of immune cells to the region increases the likely encounters between phages and immune cells that could ultimately trigger immune signaling.

Conclusion

The gastrointestinal mucosal barrier provides structure for interaction between microbiome constituents and the metazoan immune system. The organismal relationships in this space are critical for localized and systemic health and warrant further investigation. Bacteriophages have the ability to greatly impact immune responses in this locale, directly or indirectly, through regulation of the bacterial population. We are just beginning to realize the influence of the bacteriophage population, and require more experimentation using complex populations to clarify how fluctuations

may maintain health or drive dysbiosis. In this manner, we can see how elucidation of phage-bacterial interactions may result in novel therapies for gastrointestinal disorders.

Acknowledgement

This work was supported, in part, by the Winkler Bacterial Overgrowth Research Fund.

References

1. Weinbauer, Markus G. "Ecology of prokaryotic viruses." FEMS microbiology reviews 28 (2004): 127-181.
2. Mirzaei, Mohammadali Khan, and Maurice Corinne F. "Ménage à trois in the human gut: interactions between host, bacteria and phages." Nature Reviews Microbiology 15 (2017): 397-408.
3. Hobbs, Zack, and Abedon Stephen T. "Diversity of Phage Infection Types and Associated Terminology: The Problem with 'Lytic or Lysogenic'." FEMS Microbiol Lett 363 (2016): 47.
4. Sinha, Anshul, and Maurice Corinne F. "Bacteriophages: Uncharacterized and Dynamic Regulators of the Immune System". Mediators Inflamm (2019): p3730519.
5. Fraser, James S, Yu Zhou, Maxwell Karen L, and Davidson Alan R. "Ig-like Domains on Bacteriophages: A Tale of Promiscuity and Deceit." J Mol Biol 359 (2006): 496-507.
6. Liu, Minghsun, Deora Rajendar, Doulatov Sergei R, and Gingery Mari et al. " Reverse Transcriptase-Mediated Tropism Switching in Bordetella Bacteriophage." Science 295 (2002): 2091-2094.
7. Lim, Efrem S, Zhou Yanjiao, Zhao Guoyan, and Bauer Irma K et al. "Early Life Dynamics of the Human Gut Virome and Bacterial Microbiome in Infants." Nat Med 21 (2015): 1228-1234.
8. Breitbart, Mya, Haynes Matthew, Kelley Scott, and Angly Florent et al. "Viral Diversity and Dynamics in an Infant Gut." Res Microbiol 159 (2008): 367-373.
9. Pabst, Oliver, Herbrand Heike, Friedrichsen Michaela, and Velaga Sarvari et al. "Adaptation of Solitary Intestinal Lymphoid Tissue in Response to Microbiota and Chemokine Receptor CCR7 Signaling." J Immunol 177 (2006): 6824-6832.
10. Mazmanian, Sarkis K, Liu Cui Hua, Tzianabos Arthur O, and Kasper Dennis L. "An Immunomodulatory Molecule of Symbiotic Bacteria Directs Maturation of the Host Immune System." Cell 122 (2005): 107-118.
11. An, Dingding, Oh Sungwhan F, Olszak Torsten, and Zeissig Sebastian et al. " Sphingolipids from a Symbiotic Microbe Regulate Homeostasis of Host Intestinal Natural Killer T Cells." Cell 156 (2014): 123-133.
12. Boullier, Séverine, Tanguy Myriam, Kadaoui Khalil A, and Caubet Cecile et al. "Secretory IgA-mediated Neutralization of Shigella Flexneri Prevents Intestinal Tissue Destruction by Down-Regulating Inflammatory Circuits." J Immunol 183 (2009): 5879-5885.
13. Fukuda, Shinji, Toh Hidehiro, Hase Koji, and Oshima Kenshiro et al. " Bifidobacteria Can Protect From Enteropathogenic Infection Through Production of Acetate." Nature 469 (2011): 543-547.
14. Kamada, Nobuhiko, Chen Grace Y, Inohara Naohiro, and Nunez Gabriel. " Control of Pathogens and Pathobionts by the Gut Microbiota." Nature Immunology 14 (2013): 685-690.
15. Wang, Gang, Huang Shuo, Wang Yuming, and Cai Shuang. "Bridging Intestinal Immunity and Gut Microbiota by Metabolites." Cell Mol Life Sci 76 (2019): 3917-3937.
16. Kim, Chang H. "Immune Regulation by Microbiome Metabolites." Immunology 154 (2018): 220-229.
17. Lobionda, Stefani, Sittipo Panida, Kwon Hyog young, and Lee Yun Kyung. "The Role of Gut Microbiota in Intestinal Inflammation with Respect to Diet and Extrinsic Stressors." Microorganisms 7 (2019): 271.
18. de Kivit, Sander, Tobin Mary C, Forsyth Christopher B, and Keshavarzian Ali et al. "Regulation of Intestinal Immune Responses through TLR Activation: Implications for Pro- and Prebiotics." Front Immunol 5 (2014): 60.
19. Reyes, Alejandro, Haynes Matthew, Hanson Nicole, and Angly Florent E et al. "Viruses in the faecal microbiota of monozygotic twins and their mothers." Nature 466 (2010): 334-338.
20. Minot, Samuel, Sinha Rohini, Chen Jun, and Li Hongzhe et al. "The Human Gut Virome: Inter-Individual Variation and Dynamic Response to Diet." Genome Res 21 (2011): 1616-1625.
21. Knowles, B, Silveira CB, Bailey BA, and Barott K et al. "Lytic to temperate switching of viral communities." Nature 531 (2016): 466-470.
22. Arumugam, Manimozhiyan, Raes Jeroen, Pelletier Eric, and Paslier Denis L et al. "Enterotypes of the human gut microbiome." Nature 473 (2011): 174-180.
23. Keen, Eric C, and Dantas Gautam. "Close Encounters of Three Kinds: Bacteriophages, Commensal Bacteria, and Host Immunity." Trends Microbiol 26 (2018): 943-954.
24. Kim, Min-Soo, and Bae Jin-Woo. "Lysogeny is prevalent and widely distributed in the murine gut microbiota." The ISME Journal 12 (2018): 1127-1141.
25. Barr, Jeremy J, Auro Rita, Furlan Mike, and Whiteson Katrine L. "Bacteriophage Adhering to Mucus Provide a Non-Host-Derived Immunity." Proc Natl Acad Sci U S A 110 (2013): 10771-10776.
26. Joiner, Kevin L, Baljon Arlette, Barr Jeremy, and Rohwer Forest et al. "Impact of bacteria motility in the encounter rates with bacteriophage in mucus." Scientific Reports 9 (2019): 16427.
27. Kumar, Himanshu, Kawai Taro, and Akira Shizuo. "Pathogen Recognition by the Innate Immune System." Int Rev Immunol 30 (2011): 16-34.
28. Levy, Maayan, Thaiss Christoph A, and Elinav Eran. "Metabolites: Messengers Between the Microbiota and the Immune System." Genes Dev 30 (2016): 1589-1597.
29. Xiong, Xin, Zhang Hong-Mei, Wu Ting-Ting, and Xu Li et al. "Titer Dynamic Analysis of D29 Within MTB-infected Macrophages and Effect on Immune Function of Macrophages." Exp Lung Res 40 (2014): 86-98.
30. Gaubin, Muriel, Fanutti Cristina, Mishal Zohar, and Durrbach Antoine et al. "Processing of Filamentous Bacteriophage Virions in Antigen-Presenting Cells Targets Both HLA Class I and Class II Peptide Loading Compartments." DNA Cell Biol 22 (2003): 11-18.
31. Van Itallie, Christina M, Holmes J, Bridges A, and Gookin JL et al. "The Density of Small Tight Junction Pores Varies Among Cell Types and Is Increased by Expression of claudin-2." J Cell Sci 121 (2008): 298-305.
32. Nguyen, Sophie, Baker Kristi, Padman Benjamin S, and Patwa Ruzeen et al. "Bacteriophage Transcytosis Provides a Mechanism To Cross Epithelial Cell Layers." mBio 8 (2017): 1-15.
33. Carroll-Portillo, Amanda, and Lin Henry C. "Bacteriophage and the Innate Immune System: Access and Signaling." Microorganisms 7 (2019): 625.
34. Zeng, MY, Inohara N, and Nunez G. "Mechanisms of Inflammation-Driven Bacterial Dysbiosis in the Gut." Mucosal Immunol 10 (2017): 18-26.
35. Pickard, Joseph M, Zeng Melody Y, Caruso Roberta, and Núñez Gabriel. "Gut Microbiota: Role in Pathogen Colonization, Immune Responses and Inflammatory Disease." Immunol Rev 279 (2017): 70-89.
36. Sokol, H, Seksik P, Furet J. P, and Firmesse O et al. "Low Counts of Faecalibacterium Prausnitzii in Colitis Microbiota." Inflamm Bowel Dis 15 (2009): 1183-1189.
37. Cornuault, Jeffrey K, Petit Marie-Agnes, Mariadassou Mahendra, and Benevides Leandro et al. "Phages Infecting Faecalibacterium Prausnitzii Belong to Novel Viral Genera That Help to Decipher Intestinal Viromes." Microbiome 6 (2018): 65.
38. Parada Venegas, Daniela, De la Fuente Marjorie K, Landskron Glauben, and González Maria Julieta et al. "Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases." Front Immunol 10 (2019): 277.
39. Porayath, Chandni, Salim Amrita, Palillam Veedu Archana, and Babu Pradeesh et al. "Characterization of the bacteriophages binding to human matrix molecules." International Journal of Biological Macromolecules 110 (2018): 608-615.
40. Lehti, Timo A, Pajunen Maria I, Skog Maria S, and Finne Jukka. "Internalization of a polysialic acid-binding Escherichia coli bacteriophage into eukaryotic neuroblastoma cells." Nature Communications 8 (2017): 1915.
41. Shan, Jinyu, Ramachandran Ananthi, Thanki Anisha M, and Vukusic Fatima B.I. et al. "Bacteriophages Are More Virulent to Bacteria With Human Cells Than

They Are in Bacterial Culture; Insights From HT-29 Cells." Scientific Reports 8 (2018): 5091.

How to cite this article: Carroll-Portillo, Amanda; Lin, Henry C. "Bacteriophage at the Gastrointestinal Mucosal Barrier and Regulation of Mucosal Immunity". J Immuno Biol 5 (2020) doi:10.37421/jib.2020.5.145