Immunological and Microbial Responses to Bacteriophage Therapy Targeting Vancomycin-Resistant Enterococcus colonization

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Abstract

Multidrug resistant bacteria are an emerging global threat with an urgent need for alternative therapies. Bacteriophage therapy is a promising treatment for antibiotic-resistant bacteria with clinical trials currently underway. Although bacteriophage therapy has been well-studied in recent years, the overall effects of administration of this biologic on both the host and microbial responses to the subjects remain to be elucidated. While previous studies have shown production of antibodies against bacteriophage treatment, many questions remain on the role of the cellular immune response and any consequences of this response on multiple treatments within the same individual Here we describe our preliminary studies assessing immunological changes following phage administration using a mouse model of bacteriophage therapy that targets vancomycin-resistant enterococcus (VRE). To assess immune responses, we developed a high-parameter flow cytometry panel to investigate the recruitment and phenotype of the innate and adaptive immune cell compartment post bacteriophage therapy. We also collected serum samples to measure antibody production against bacteriophage in our model. Flow cytometry analysis shows similar frequencies of innate cells after primary phage treatment including populations of dendritic cells and macrophages. After secondary exposure of phage, mice that were treated with bacteriophage have different frequencies of lymphocyte populations compared to mice treated with vehicle water including increased B cells and reduced T cells. Mice treated with phage also showed an increased population of activated CD8⁺ T cells. Lastly, mice treated with primary phage showed production of anti-phage antibodies with increased production after secondary phage treatment. These data indicate that bacteriophage therapy does induce an immune response and future studies will further investigate the mechanism governing this response. A better understanding of the immune response that occur to bacteriophage therapy could aid future advances of this treatment.

Introduction

- Vancomycin resistant enterococcus (VRE) is a prevalent bacteria detected in the microbiome with 40% of ICU patients and nursing home residents colonized (Davis et al)
- VRE can become life threatening if it infiltrates the bloodstream and causes systemic infection.
- Bacteriophage (phage) therapy is a potential alternative treatment to antibiotics that utilizes viruses that specifically kill target bacteria
- Phage therapy has largely been used for compassionate use, but it use is currently ongoing in clinical trials
- It is unclear what role the immune system plays during phage therapy and if immunity to phage prevents reuse
- Previous studies have reported production of anti-phage antibodies, but it is unknown what the cellular and molecular mechanism eliciting these responses.

What are the cellular and molecular immune responses to phage therapy?

Does the host generate antibodies against phage and are they neutralizing?

Materials and Methods

Murine model to assess immune response to primary and secondary phage treatment

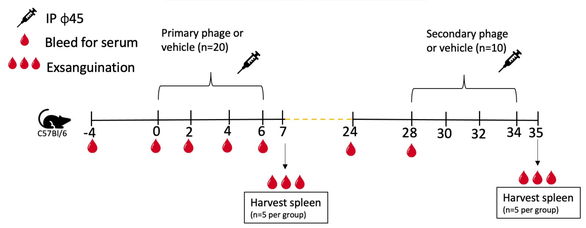


Figure 1. Wildtype C57Bl/6 mice were treated with phage 45 (10^9 PFU/day), a siphoviridae lytic phage that targets VRE, or water vehicle by intraperitoneal (IP) injection for 7 days. Half the mice were euthanized day 7 to harvest spleen and sera. The remaining mice received a secondary course of phage or water treatment with euthanasia on day 35 also to acquire spleens and sera. Harvested spleens were used for flow cytometry experiments and sera was used in ELISAs to detected antibody responses.

Flow cytometry panel to investigate innate and adaptive immune responses

Fluorophore	Antigen	Function
BUV395	CD62L	Naïve T cell
BUV737	CD80	Co-stimulatory molecule
BV421	CD3	T cell receptor
BV480	L/D	Live/dead
BV605	CD69	T cell activation
BV711	NK1.1	NK cells
BV785	CD19	B cells
AF488	CD11b	Myeloid cells
PercpCy5.5	CD44	Memory T cells
PE	F4/80	Macrophages
PE-CF594	CD4	Helper T cells
PE-Cy7	CD11c	DC marker
AF647	MHC-II	Hemotopoitic cells
AF700	CD45	Molecule for antigen presentation
APC-H7	CD8	Cytotoxic T cells

Table 1. List of antibodies in flow cytometry experiments staining murine spleens on day 7 (primary phage) and day 35 (secondary phage) harvests.

Detecting anti-phage antibody responses with ELISA

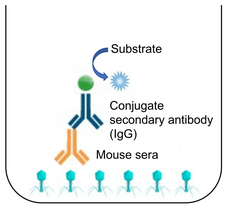


Figure 2. Diagram outlining indirect ELISA used to assess IgG antibody responses against phage. Phage 45 was coated on plates as antigen at a $5x10^7$ PFU/well concentration. Mouse sera was used a primary antibody, while IgG was used a secondary to quantify and characterize anti-phage antibodies.

Results and Discussion

Similar frequencies and phenotype of splenic innate cells after primary exposure of phage

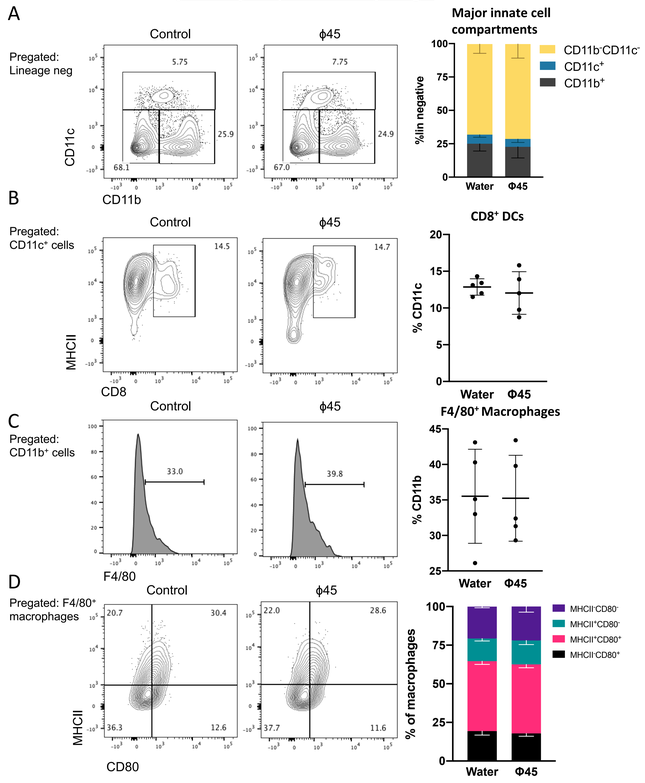
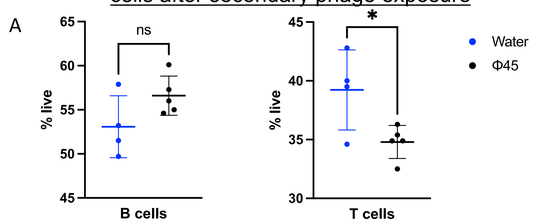


Figure 3. Splenic cells were harvested one day after a 7-course treatment of phage therapy or water for flow cytometry analysis. Similar frequencies of (A) major innate populations (CD11b, CD11c, and CD11b⁻CD11c⁻) (B) CD8⁺ dendritic cells and (C) macrophages between phage and water treatment. (D) Macrophages between groups also showed similar expression of markers of antigen presentation (MHCII) and activation (CD80). (n= 5 per group)

Decreased T cell frequencies and increased activated CD8⁺ T cells after secondary phage exposure



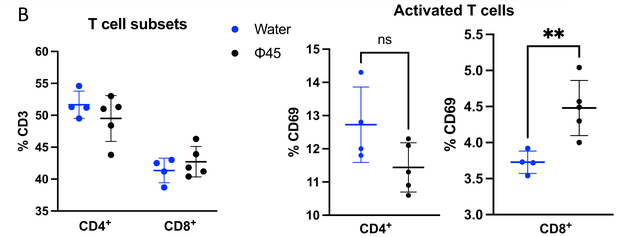


Figure 4. Splenic cells were harvested day 35 after a secondary phage or water treatment and analyzed using flow cytometry. (A) Mice that received phage show a trend for increased B cell frequencies and a significant decrease in T cell populations. (B) Further analysis of T cell populations show similar T cell subsets with an increased CD8⁺ T cell activation not seen with the CD4⁺ T cells. (*p <0.05 ** p< 0.01 n= 5 per group)

Detection of anti-phage IgG antibodies post primary and secondary exposure to bacteriophage therapy

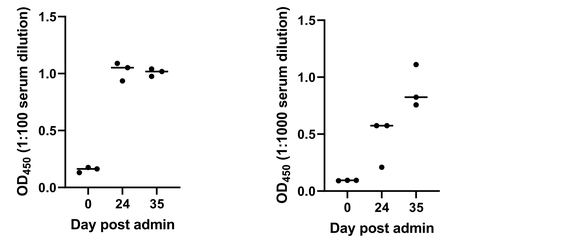


Figure 5. Sera was collected from mice before treatment (D0), after primary phage treatment (D24), and after secondary phage treatment (D35). Sera was tested for the presence of anti-phage antibodies using indirect ELISA outlined in Figure 2. Increased absorbance at dilutions 1:100 and 1:1000 of sera suggest the presence of anti-phage antibodies after phage treatment. The 1:1000 dilution showed increased absorbance after a secondary phage treatment compared to primary. (n=3 per group)

Summary

- Mice were treated with primary and secondary phage treatment compared to vehicle water and then spleen and sera were assessed for cellular immune changes and anti-phage antibody production
- No significant differences were observed in the recruitment of innate populations assessed (including dendritic cell and macrophage populations) after primary or secondary phage treatment
- After secondary phage exposure, T and B cell frequencies cells shifted indicating a potential expansion of B cells compared to vehicle control
- Anti-phage antibodies were detected after primary phage exposure and enhanced with secondary phage treatment.
- Future studies will dissect what is the cellular immune response governing antibody production and if anti-phage antibodies are neutralizing.