# Examining phage immunogenicity: Towards safer and more effective bacteriophage therapy

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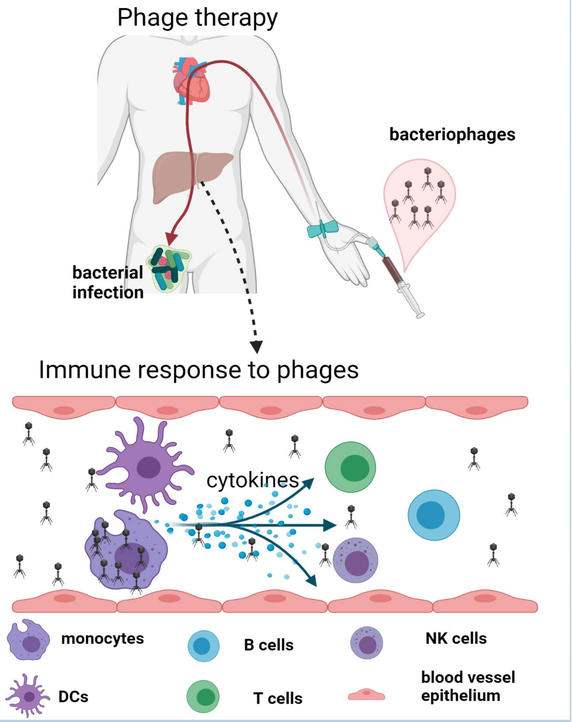
## Abstract


**Key findings**

- Bacteriophages (phages) are engulfed primarily by monocytes in vitro
- Endotoxin free phages can trigger inflammatory responses that are based on the phage species


## Introduction

- Multidrug resistant (MDR) infection is one of today's biggest threats to global health. In US, there are 2.8 millions antibiotic-resistant infections that kill 35.000 people each year[1].
- As bacteriophages naturally infect bacteria, phage therapy is becoming a promising treatment to MDR infections with several successful cases reported[2]. In Australia, phage therapy is only available through clinical trials, one of which is at Westmead Hospital[3].
- Phage cocktails are personalized based on the MDR bacterial strains isolated from the patients[4].
- Phages can be cleared from circulation within hours of treatment. How this occurs in humans, and whether there is a resulting immune/inflammatory response has not been characterized[2].

## Results

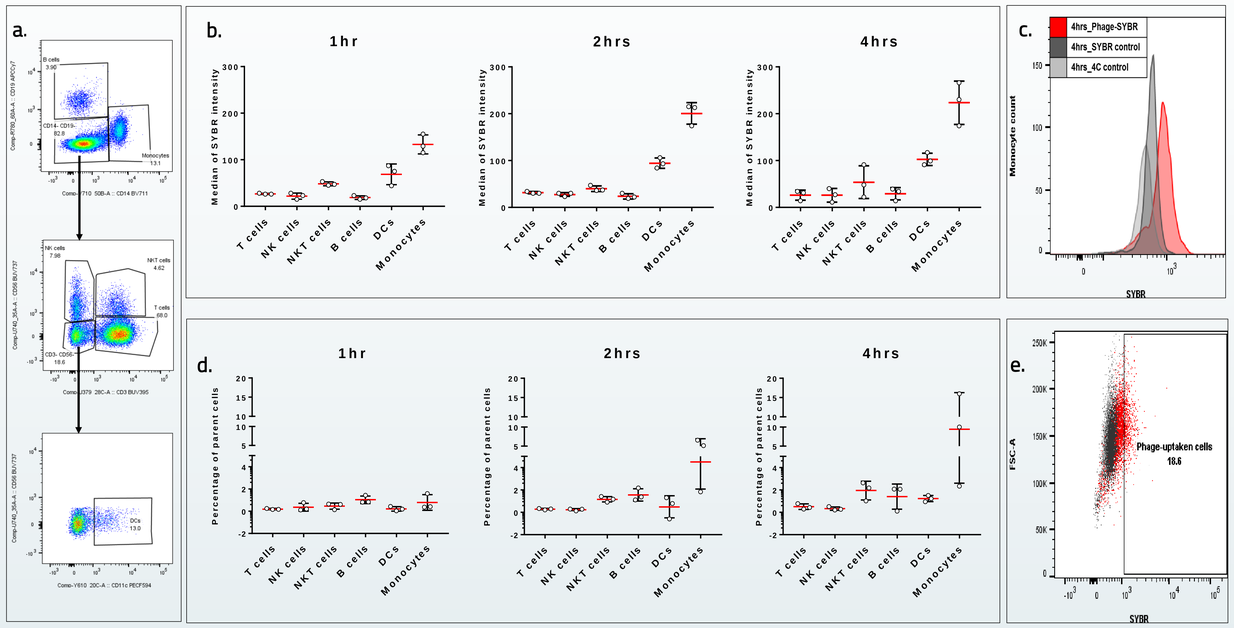



Figure1: Flow cytometry analysis of PBMCs treated with SYBR green-stained phages over 4 hours. **a.** Flow cytometry gating to identify cell types. **b.** SYBR green median fluorescence intensity (MFI) stratified by cell population following 1, 2 or 4 hours of incubation. **c.** Monocyte SYBR fluorescence histogram following treatment with SYBR-tagged phages, SYBR control and SYBR-phages at 4C. **d.** Proportion of immune cell types engulf phages after 1-2-4 hours. **e.** Overlaid density plot of monocytes with gating fraction of cells that engulf phages. SYBR, Gold SYBR-green. NKT cells, natural killer T cells. DCs, dendritic cells. n=3.

- Phages are engulfed primarily by monocytes and Dendritic cells (DCs).
- Phage uptake occurs quickly, within the first hour and increases up to 4 hours.
- Up to 15% of monocytes take up a detectable-level of SYBR-tagged phages after 4 hours.

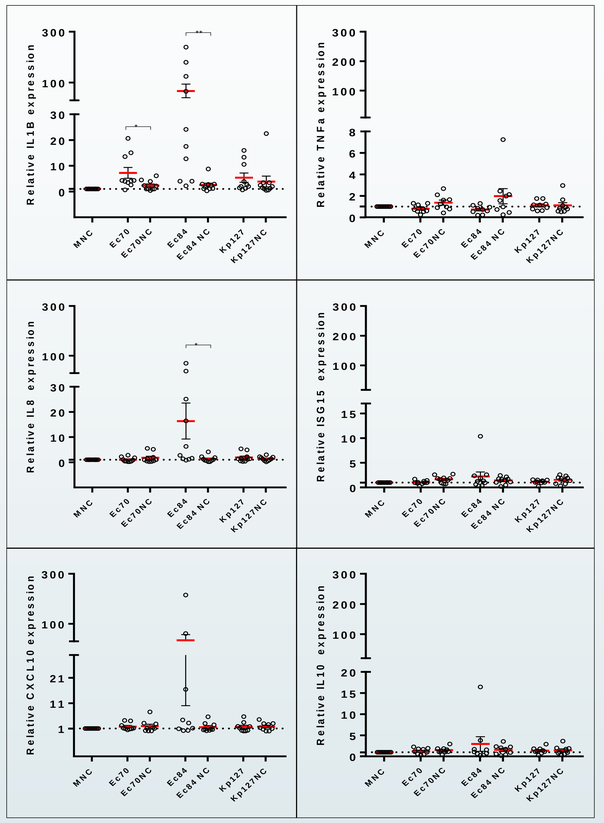



Figure 2: qPCR analysis of PBMC cytokine expression upon treatment with different phage families. MNC, media only negative control. Ec70NC/Ec84NC/Kp127NC, phage-filtered media control. n=10, Wilcoxon test, * p<0.05, ** p<0.01.

- Some phages (Ec70, Ec4 but not Kp127) stimulate induction of certain inflammatory cytokines ( IL1b, IL8 and CXCL10 but not TNF). The level of induction varies significantly among participants suggesting that history of phage exposure may dictate responses.
- These phage species do not activate interferon-stimulated gene expression (ISG15), nor do they stimulate the expression of the anti-inflammatory cytokine, IL10.

## Methodology

- Peripheral blood mononuclear cells (PBMCs) were cultured with 3 different phage families: Myoviridae (Eco70), Podoviridae (Ec84), Siphoviridae (Kp127) with an FDA-approved endotoxin level (< 5EU/ml), multiplicity of infection MOI =10 (phages: cell).
- Phages were labelled with SYBR green. Excess SYBR was removed by washing using a 100kDa cut-off membrane and phages were incubated with PBMCs for up to 4 hours. Controls include a filtered SYBR control (SYBR washed without phage) and 4C co-cultures that prevent phage engulfment.
- To assess immune responses to individual phage species, PBMCs were treated with 3 phage species for 24 hrs. Cytokine expression was measured by qPCR, normalized to the 18S housekeeper. To generate phage-specific negative controls, phages were removed from the preparation using a 100kDa membrane and the filtrate was used to treat PBMCs.

## Conclusion

- **The immune/inflammatory responses to phages require significant study to ensure the safety of phage therapy in the future.**
- **Less immunogenic phages should be selected or developed to ensure better outcomes.**
- **Inhibition of phage engulfment by monocytes has the potential to increase circulating phage titers and improve the efficiency of therapy.**

## References

1.CDC's Antibiotic Resistance Threats in the United States, 2019. 2. Aslam S et al. Open Forum Infect disease. 2019. 7(9). 3. Petrovic FA et al. Nat Microbiology. 2020.5(3). 4. Schooley RT et al. Antimicrobial Agents and Chemotherapy 2017.61(10).

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