Chemistry in Cells DPhil Programme
Project proposal 2023

Innate immune cell activation to kill antimicrobial resistant bacteria.

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Abstract

We have been using chemical biology approaches to study how stimulation of the orphan G protein Coupled Receptor (GPCR) GPR84 can enhance the ability of innate immune cells called macrophages to capture and kill bacteria. Using novel small molecule GPR84 agonists we can enhance macrophage phagocytosis and killing of live *E.coli* bacteria. The goal of this project is to extend our GPR84 studies and screen for other chemicals that can enhance the uptake and killing of antibiotic resistant bacteria in tissue culture assays before testing their activity as innate immune adjuvants *in vivo.*

Introduction

Antibiotics are drugs that kill bacteria which cause life-threatening infections. The successful use of antibiotics in medicine is threatened by the development of antimicrobial resistance (AMR). The World Health Organisation (WHO) estimate that more than one million people a year die from infections that resist treatment with antibiotics. Very few new classes of antibiotics have been developed over the past 30 years. One alternative approach to treat bacterial infections is to develop new immune adjuvant therapies in which drugs are used to stimulate the host immune response. Most immune adjuvants enhance the immune response to tumours but in this project we plan to stimulate macrophages, a key cell type of the innate immune response, to phagocytose and kill AMR bacteria. An important advantage of targeting the host rather than the pathogen is that this would remove selective pressure for evolution of microbial resistance.

Work leading up to the project

We have shown that a range of small chemicals that activate the GPR84 receptor can enhance macrophage phagocytosis of labelled bacteria (e.g. Ref 1 = Recio et al, 2018). In recent experiments in collaboration with a bacteriology lab led by Prof Tang we have started to study how different GPR84 agonists affect macrophage phagocytosis of live bacteria using gentimycin protection assays. Figure 1 shows that treatment of the human THP-1 monocyte/ macrophage cell line with small chemicals enhances the phagocytosis and killing of a non-pathogenic strain of E.coli (DH5alpha). Medicinal chemistry approaches to identify new GPR84 agonists remains an important approach to take this project forward.

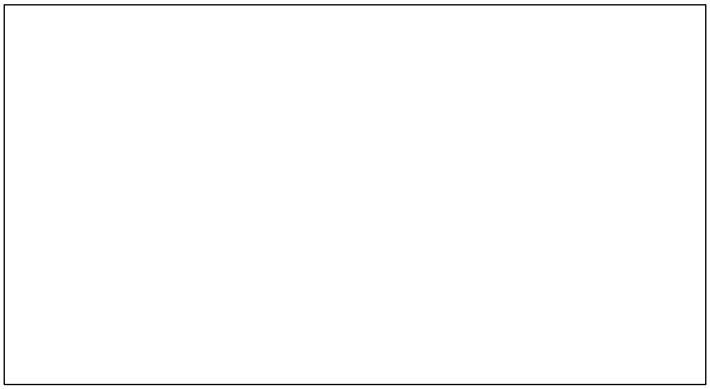

Figure 1. *The number of viable E coli bacteria recovered from differentiated THP-1 cells in a gentimicin protection assay is plotted following treatments with a number of GPR84 agonists.*

Control experiments include XXX, XXX and XXX. Data is represented as mean +/- standard deviation. Similar results have been obtained in over 10? independent series of experiments

Proposed methods

We currently follow macrophage phagocytosis using three different methodologies; live cell imaging using pH sensitive killed bacteria, confocal microscopy of macrophages incubated with bacteria and specific antibodies and gentamicin protection assay. Students joining this project will use chemical biology approaches to optimise our innate immune adjuvant experiments. After initial training in how to perform macrophage phagocytosis assays this project could be extended in different directions including; 1) screening the effect of existing and new GPR84 agonists against a wider range of bacterial species including AMR bacterial pathogens; 2) screening collections of small molecules for their ability to enhance macrophage phagocytosis of bacteria and 3) using chemical probes to study the mechanism by which GPR84 agonists enhance macrophage phagocytosis.

References

1 Recio C., et al., *Frontiers in Immunology*, 2018, **40**, 1234
2 Lucy D., et al., *ACS Chemical Biology*, 2019, **40**, 1234
3 Pinqi et al., Journal of Medicinal Chemistry, 2023, under review

Supervisory team

Appropriate supervision arrangements need to be in place for the duration of the DPhil project. All primary and secondary supervisors listed should be qualified to supervise a DPhil (not senior PDRAs). Please indicate the following:

1. Proposed Physical Sciences Supervisor
(i) Name, dept, email:
Angela Russell, Depts Chemistry and Pharmacology

I am an experienced supervisor whose contract could potentially finish before the end of the substantive DPhil project: **No**
I have supervised fewer than two DPhil students to completion: **No**

(ii) Laboratory where student will be based, and estimate % of time in that lab (if more than one lab, please specify):
CRL ~40%

(iii) Day-to-day supervisor if different from above (*e.g.* senior PDRA):
Supervision and training in all aspects of safe handling of bacterial species will be provided by Dr Rachel Exley and PDRAs in the Tang laboratory.

(iv) Name and department of secondary supervisor (in the event that the supervisor listed in 1(i) can no longer supervise):
Current biological supervision of our work on

2. Proposed Biomedical Sciences Supervisor
(i) Name, dept, email:
David R. Greaves and Christoph Tang

I am an experienced supervisor whose contract could potentially finish before the end of the substantive DPhil project: **No**
I have supervised fewer than two DPhil students to completion: **No**

(ii) Laboratory where student will be based, and estimate % of time in that lab (if more than one lab please specify):
Greaves & Tang labs

(iii) Day-to-day supervisor if different from above (*e.g.* senior PDRA):

(iv) Name and department of secondary supervisor (in the event that the supervisor listed in 2(i) can no longer supervise):
Dr Rachel Exley (Tang Lab)

3. Proposed additional collaborators, including industrial collaborators (optional):

Name, institution/department, and e-mail address

Positive research culture

By submitting a full project proposal, ALL supervisors must agree to adhere to the positive research culture policy as outlined by the Wellcome Trust in their positive research culture web-pages: https://wellcome.ac.uk/what-we-do/our-work/research-culture. This includes *"What researchers think about the culture they work in"* report which can be downloaded: https://wellcome.ac.uk/sites/default/files/what-researchers-think-about-the-culture-they-work-in.pdf

☒ I have read the Wellcome Trust positive research culture webpages including the *"What researchers think about the culture they work in"* report and agree to adhere to a positive research culture policy. *Failure to adhere to the positive research culture policy may lead to removal of funding and supervisors being excluded from future calls for projects.*