Department of Veterinary Medicine: Funded PhD

Project Title: VHHs for the prevention and control of Campylobacter spp

Supervisor: Dr Andrew Grant

Supervisor profile page: Dr Andrew Grant | Department of Veterinary Medicine

Applications are invited for an AstraZeneca funded non-clinical PhD awarded to the University of Cambridge to be led by Dr Andrew Grant at the Department of Veterinary Medicine. The successful applicant will spend time at the AstraZeneca R&D site in Cambridge as well as the Department of Veterinary Medicine, according to the capacities and/or expertise at each site relevant to the different aspects of the project.

Background: Campylobacter spp. are the most prevalent cause of bacterial diarrhoeal disease. There is an urgent need to develop novel therapeutic options.

Concept: We contend that there is an exciting opportunity to employ and develop our VHH single-domain antibody library capabilities, to isolate binders, and inhibit the activity of, key surface-exposed proteins essential for Campylobacter jejuni and Campylobacter coli viability or infection. The use of VHH domain libraries will provide increased selection design flexibility, which will enable us to by-pass immunodominance and enrich for binders that target low abundance and conserved antigenic determinants. Moreover, the small size of the VHH domain and its characteristic paratope structure enables epitopes that are not usually accessible to conventional antibodies to be targeted. The lower complexity structure of VHHs, compared to full-length antibodies, also enables creative solutions for their production and delivery.

Aim: To identify and exploit VHH single-domain antibodies that have bactericidal/bacteriostatic activity and, if possible, are independent of the immune system. We plan to use VHHs, to bind to surface-exposed proteins of Campylobacter that are essential for viability and/or colonisation. We will also employ a complementary hypothesis-free strategy to identify VHH with antimicrobial properties.

Objective 1: Isolation of a sequence diverse panel of VHHs against C. jejuni and C. coli recombinant proteins and whole bacteria. We have prioritised antigens that are core C. jejuni and C. coli extracellular or outer membrane proteins that are either ‘essential’ (required for fitness) or ‘conditionally essential’ (required for colonisation). We plan to screen VHH libraries against the recombinant proteins as well as a complementary hypothesis-free, target-independent, strategy to identify binders with antimicrobial properties, for this we will use whole C. jejuni and C. coli. By performing multiple rounds of affinity selection against the antigens and the whole bacteria, a population of single-domain antibodies that can access the antigenic target on the bacteria will be isolated.

Objective 2: Screens for anti-Campylobacter VHH functional activity. VHHs will subsequently be ranked by potency in cell-based in vitro assays and if necessary, affinity, as determined by Biacore-led surface plasmon resonance measurements. From the
enriched population, we will use Sanger sequencing to obtain sequence data for the hits of interest. Unique VHHs will be screened via in vitro assays (ELISA) for their ability to bind to C. jejuni and C. coli in a strain-independent manner. This will triage the VHHs to enable us to select clones for further evaluation; these being VHH that affect the growth rate or viability of C. jejuni and/or C. coli. We will examine the target/epitope landscape of the VHH panel by competition ELISA and epitope binning using Octet Bio-layer interferometry. Subsequently, for the target-independent screens, the target proteins for the functional VHHs will be determined using immunoprecipitation and mass spectrometry techniques.

Objective 3: Construction of biological delivery systems for anti-Campylobacter single-domain antibodies. Following library screening, the DNA pool representing the selected VHH population will be recovered, and full-length open reading frames will be cloned into a suitable vector and introduced into different harmless bacteria, and/or live attenuated vaccine strains, to produce VHHs against C. jejuni and/or C. coli antigens. The biological activity of these VHHs will be analysed by ELISA, agglutination assays and cell viability assays and assessed in in vitro co-culture experiments.

Expected outcome: We aim to identify a large repertoire of VHHs from target-directed and target-independent screens to determine whether binders targeted to essential proteins of a bacterial pathogen can display bacteriostatic and bactericidal effects. We will explore creative solutions to engineer harmless bacteria and/or live attenuated vaccine strains to produce or release the VHH in situ for therapeutic and/or prophylactic applications.

Training: The project will provide training and practical experience in handling and manipulating bacteria, molecular biology, protein purification, high throughput screening, bioinformatics, imaging, proteomics techniques and immunological assays.

For further information about the project, please contact Dr Andrew Grant.

Funding: This project is funded by an AstraZeneca Non-Clinical studentship awarded to the University of Cambridge. Funding is for 3.5 years and will cover tuition fees (at the UK rate), stipend and allocation toward project costs and training.

How to apply: Applicants must submit a formal application by the 31st January. Shortlisted candidates will be contacted in mid February and interviews will be conducted on the 1st March.

Contact the Supervisor (ajg60@cam.ac.uk) to discuss the project before submitting an official application.

More here on application process here: PhD in Biological Sciences at the Department of Veterinary Medicine | Postgraduate Admissions (cam.ac.uk)