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Using the local immune response from the natural buffalo host to generate an antibody fragment library that binds the early larval stages of *Schistosoma japonicum*

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ABSTRACT

Antibodies isolated from the local draining inguinal lymph node of field exposed-water buffaloes following challenge with *Schistosoma japonicum* cercariae showed high reactivity towards *S. japonicum* antigen preparations and bound specifically to formaldehyde-fixed *S. japonicum* schistosomules. Using this specific local immune response we produced a series of single-chain antibody Fv domain libraries from the same lymph nodes. Removal of phage that cross reacted with epitopes on adult parasites yielded a single-chain antibody Fv domain-phage library that specifically bound to whole formaldehyde-fixed and live *S. japonicum* schistosomules. DNA sequencing indicated clear enrichment of the single-chain antibody Fv domain library for buffalo B-cell complementarity determining regions post-selection for schistosomule binding. This study also revealed that long heavy chain complementarity determining regions appear to be an important factor when selecting for antibody binding fragments against schistosomule proteins. The selected single-chain antibody Fv domain-phage were used to probe a schistosome-specific protein microarray, which resulted in the recognition of many proteins expressed across all schistosome life-cycle stages. Following absorption to adult worms, the single-chain antibody Fv domain-phage library showed significantly reduced binding to most proteins, whilst two proteins (NCBI GenBank accession numbers AY915878 and AY815196) showed increased binding. We have thus developed a unique set of host derived single-chain antibody Fv domains comprising buffalo B-cell variable regions that specifically bind to early *S. japonicum* life-stages.

1. Introduction

Despite many years of significant effort and expenditure, *Schistosoma japonicum* infection remains a serious threat to public health in the People's Republic (PR) of China with more than 30 million people, living in tropical and subtropical regions, at risk of disease (Ross et al., 2001). Complicating control efforts for *S. japonicum* is the fact that this species can infect over 40 species of wild and domestic animals and zoonotic transmission is prevalent with animals acting as major reservoirs of infection (McManus and Bartley, 2004; Lin et al., 2011; Liu et al., 2012). In 2001 it was estimated that 865,000 people and over 100,000 bovines were infected by *S. japonicum* in provinces where the disease is endemic (Ross et al., 2001). Bovines, particularly water buffaloes (*Bubalus bubalis*), are the major reservoir responsible for disease transmission to humans (Li et al., 2014). Mathematical modelling has estimated that 75% of animal to human transmission can be directly attributed to buffaloes in marshland areas (Williams et al., 2002). Whilst adding to the complexity of programs aimed at controlling schistosomiasis japonica, the zoonotic nature of transmission may provide novel approaches to develop vaccines that prevent human

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infection by targeting the major animal reservoirs (McManus and Bartley, 2004).

It is generally accepted that effective control regimes will benefit greatly by incorporating efficacious vaccines with existing or alternative therapies (McManus and Loukas, 2008; Lin et al., 2011). Vaccination with UV-attenuated *S. japonicum* cercariae has been successful in generating protection in a number of animal models including pigs (Lin et al., 2011) and water buffalo (Shi et al., 1990), indicating effective immunity against the early life-stages can be generated. Unfortunately, pursuing an irradiation approach to control schistosome infections has proven impractical for large scale application (Abath, 2000; Lin et al., 2011). A number of conventional single antigen vaccine candidates have been identified, including Sm28 (Balloul et al., 1987), triosephosphate isomerase (TPI) (Harn et al., 1992) and SM23 (Reynolds et al., 1994). A combined DNA and protein vaccine, SjCTPI-Hsp70, is being evaluated as part of an integrated schistosome control trial in China (Gray et al., 2014). However, as yet, no antigen has induced sufficient protection to generate an effective commercial vaccine (Bethony et al., 2011).

Combinatorial bacteriophage libraries displaying single-chain antibody Fv domains (scFvs) have proven to be useful reagents in the identification of antibodies directed towards defined target antigens (Winter et al., 1994). As occurs in natural immunity, antibody variable regions can be subjected to random maturation within phage particles, resulting in the selection of mutants with higher binding affinities (Winter et al., 1994). Phage display technology has been used to investigate antibody interactions specific to a diverse range of disease conditions affecting a number of species including humans (Kelly et al., 2008), sheep (Maass et al., 2007), rabbits (Bridges et al., 1996) chickens (Davies et al., 1995) and cattle (Koti et al., 2010).

Research has shown that bovine antibodies have the longest heavy chain complementarity determining region 3 (CDRH3) of any known mammal antibody, with ultra-long subsets ranging in lengths of up to 61 amino acids (Wang et al., 2013). In human antibodies, long CDRH3 regions have been pivotal in the successful defence against dangerous infections such as HIV (Doria-Rose et al., 2014); however in a bovine setting the functional mechanisms for ultra-long CDRH3 regions have yet to be elucidated (Wang et al., 2013). Bovines provide an exceptional source of large lymph nodes rich in B-cells from which diverse scFv libraries can be generated representing the local variable antibody gene repertoire following a parasite infection. In this study, we describe the generation of a scFv-phage library encoding B-cell heavy chain variable region (VH) and light chain variable region (VL) gene families from the local draining lymph node of *B. bubalis* following an experimental infection with *S. japonicum* cercariae. Buffalo used in this study had previous natural exposure to *S. japonicum* as they were positive for the presence of schistosome eggs; the immune response to *S. japonicum* was then "boosted" with an experimental infection. Following selection rounds, the scFv-phage displayed long CDRH3 regions and specifically recognised proteins from the larval stage of both fixed and live *S. japonicum*.

2. Materials and methods

2.1. Parasite collection and crude protein extracts

Cercariae from *S. japonicum* were shed from infected *Oncomelania hupensis* snails collected from an endemic region in PR China using described methods (Moertel et al., 2006; Gobert et al., 2009). Cercariae were mechanically transformed and cultured into schistosomules using a modified method as described (Wang et al., 2006). Briefly, shed cercariae were transferred into chilled complete RPMI media (RPMI +L-glutamine (Gibco, Life Technologies, USA) containing 10% (v/v) foetal bovine serum (FBS; Gibco, Life Technologies)) and centrifuged at 500*g* for 5 min at 4 °C. They were then resuspended in 10 ml of warm Basch medium (containing 10% (v/v) FBS (Gibco, Life Technologies), and 4 mg/ml of cell culture penicillin streptomycin (Sigma–Aldrich, USA)). Transformation was then achieved by passing the cercariae 25 times through a 22 gauge double-ended luer lock needle. Tails were separated by swirling the cercariae in a petri dish. The schistosomules generated were then resuspended in 10 ml of Basch medium and incubated for 4 days at 37 °C with 5% CO_2.

Adult *S. japonicum* worm pairs were collected from infected mice housed in facilities located at the QMIR Berghofer Institute for Medical Research as described (Jones et al., 2007). For antigen preparations, lung-stage schistosomules and adult worms were collected as described (Gobert et al., 2009) and stored at −80 °C until required. Crude antigen was prepared from cercariae and schistosomules by resuspending the frozen parasites in lithium dodecyl sulphate (LDS) sample buffer (including DTT reducing agent; Life Technologies, USA), followed by incubation at 95 °C for 10 min and centrifugation at 12,000*g* for 10 min. Soluble adult antigen (SWAP) and soluble egg antigen (SEA) homogenates were prepared as described (You et al., 2010). Protein concentration was determined by bicinchoninic acid assay (BCA) (Thermo Fisher Scientific, USA).

2.2. Experimental animal infections and sample collection

Bovine animal experiments and *S. japonicum* sample collection were conducted in the PR of China as described (McWilliam et al., 2013c). Briefly, six mixed sex (four male, two female) *B. bubalis* were obtained from a *S. japonicum*-endemic region in Hunan Province, PR China. Animals were selected based on positive schistosome faecal egg counts indicating previous exposure to the parasite. Animals were rested for 7 weeks, during this time they were drenched with praziquantel (PZQ; 25 mg/kg), immediately upon arrival at the experimental enclosure and again in week 4. Animals were randomly assigned into two experimental groups ($n = 3$ per group). Each animal in group 1 was infected with 400 *S. japonicum* cercariae percutaneously on the inner thigh. Group two was the uninfected control group. Animals were euthanised 5–6 days p.i. Written approval for animal experiments was provided by the Ethical Review Board of the Hunan Institute of Parasitic Diseases, PR of China (approval # 110818), QIMR Berghofer Medical Research Institute Animal Ethics Committee (approval # A0108-054) and from Monash University, Australia, Animal Ethics Committee (approval # 2011-124-FW).

2.3. Collection of skin-draining lymph node samples and antibody secreting cell (ASC) antibodies

The inguinal lymph nodes (inguinal-LN) draining the inner thigh of buffaloes following experimental *S. japonicum* infection were isolated according to the method of Meeusen and Brandon (1994b). Briefly, lymph node slices were teased apart in cold RPMI media supplemented with AlbuMax II (2 mg/ml; Gibco Life Technologies, USA) under sterile conditions. Cells were washed twice with cold RPMI media (Gibco, Life Technologies, USA), and enumerated using a haemocytometer. RNA was prepared from 1×10^9 cells using QIAzol® lysis buffer and further purified using an RNeasy® Mini Kit (QIAGEN, Netherlands) according to the manufacturer's recommendations. RNA concentration was determined by absorbance at 260 nm using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA) and stored at −80 °C until required. The concentration of the remaining cells was adjusted to 5×10^6 -cells/ml in RPMI supplemented with 2 mg/ml AlbuMax II (Gibco, Life Technologies, USA). Antibodies produced by the antibody