Nanobodies targeting acetylcholinesterase as a novel intervention to control schistosomiasis

Khushi Jain[1]; Patrick J. Skelly[2], Akram Da'Darah[2]

[1]School of Arts and Sciences, Tufts University, Medford, MA, USA ;

[2]Molecular Helminthology Laboratory, Dept. of Infectious Disease and Global Health, Cummings School of Veterinary Medicine, Tufts University, North Grafton, MA, USA

Background

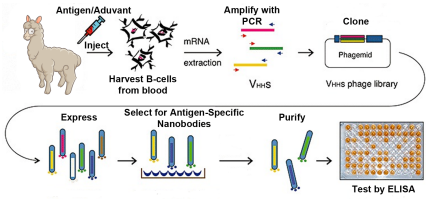
- Schistosomiasis is a neglected tropical disease caused by intravascular parasites called schistosomes that infect > 200 million people worldwide
- Schistosomiasis is contracted when mammals come in direct contact with fresh water infested with larval schistosomes (called cercariae). Within the host, cercariae transform into juvenile forms called schistosomula and these mature into adult worms in blood.
- Schistosomiasis can be treated with the drug praziquantel, However reinfection rates are high and there is concern for drug resistance. Therefore, new and improved interventions for schistosomiasis are needed and this is the subject of the research described here.

Purpose

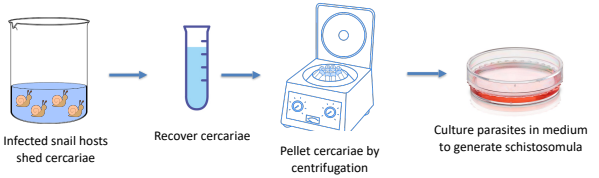
- Our laboratory has described several key proteins that are expressed on the host-exposed tegument (skin) of intravascular schistosomes
- One such protein is an **acetylcholinesterase (SmTAChE);** it is host-interactive and its expression is necessary for host infection. We have expressed this protein in the lab.
- As described below, we have generated several small antibody fragments (called nanobodies) that can bind to this SmTAChE enzyme
- **The goal of this project is to determine if some of the anti-SmTAChE nanobodies block acetylcholinesterase activity (1) on the recombinant enzyme and (2) on the surface of the parasites**
- In the longer term, such anti-SmTAChE nanobodies could be employed to treat schistosomiasis

Materials & Methods

Creating Nanobodies

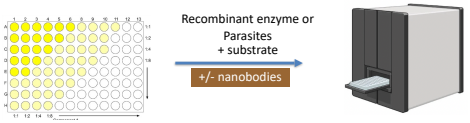


Recovering Parasites



Measuring Acetylcholinesterase Activity

Acetylcholinesterases cleave a synthetic substrate to generate a yellow colored product (left) that we detect in a microplate reader (right)

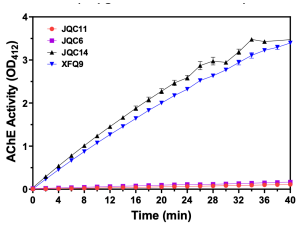


Nanobodies that block acetylcholinesterase action lead to less yellow colored product being generated in this assay

Results

1. Testing the effects of 3 different anti-SmTAChE nanobodies on the activity of the recombinant enzyme (rSmTAChE).

A.

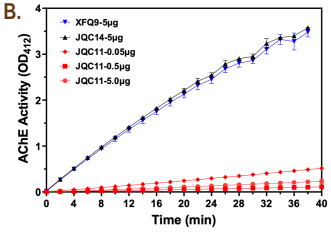


The three tested nanobodies (designated JQC11, JQQC6 & JQC14) are known to bind to SmTAChE as measured by ELISA. Nanobody XFQ9 is a control that does not bind to SmTAChE.

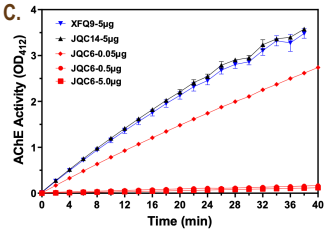
Result: (A) When tested in the enzyme assay at 10 µg, both JQC11 (red line) and JQC6 (purple line) inhibit rSmTAChE activity. Neither JQC14 (black line) nor the control XFQ9 (blue line) inhibit the enzyme.

As shown in **(B)** for JQC11 and in **(C)** for JQC6, these nanobodies are potent and only begin to lose their ability to block rSmTAChE activity at the lowest level tested (0.05 µg).

B.

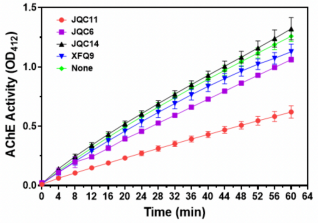


C.



2. Testing the effects of these anti-SmTAChE nanobodies on AChE activity on live schistosome parasites

A.

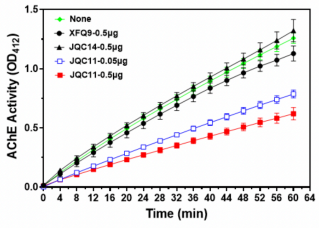


In this experiment the nanobodies are being tested against the SmTAChE that is expressed on the surface of living schistosomula.

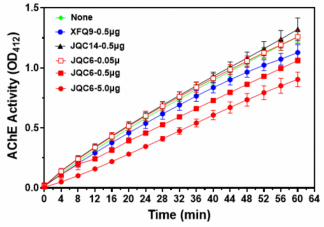
Result: (A) When tested in this assay at 10 µg, JQC11 (red line) inhibits SmTAChE activity on worms by ~ 50% compared to control (XFQ9, blue). JQC6 (purple) inhibits SmTAChE activity by ~10%. Neither JQC14 (black) nor control XFQ9 inhibit the enzyme. An additional control here is activity in the absence of any nanobody (None, green)

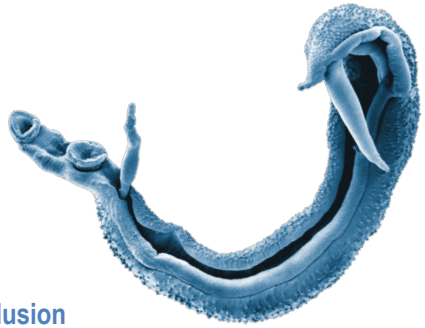
As shown in **(B)** for JQC11 and in **(C)** for JQC6, these nanobodies block SmTAChE activity on live worms in dose response – the lower the amount of nanobody added, the lower the enzyme inhibition.

B.



C.





Conclusion

We have discovered potent anti-SmTAChE nanobodies (JQC6 and JQC11) that block acetylcholinesterase activity in a dose-dependent manner when tested with either the recombinant enzyme or parasites *in vitro*. Of those tested, JQC11 is most effective at inhibiting AChE activity.

Future Directions

- When SmTAChE gene expression is knocked down by RNA interference (RNAi), worms cannot establish a robust infection in mice (Ref 1). Therefore, schistosomes need SmTAChE to survive. We predict that using the newly identified SmTAChE-blocking nanobodies will mimic this RNAi effect. Blocking SmTAChE activity on the worms is predicted to debilitate the worms and cure infection.
- Next steps involve testing the best anti-SmTAChE nanobodies to treat infection in experimental animals and, in the longer term, in people

Acknowledgements

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