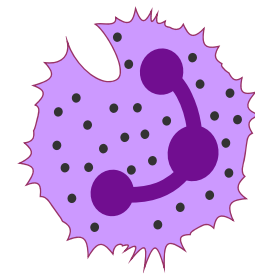




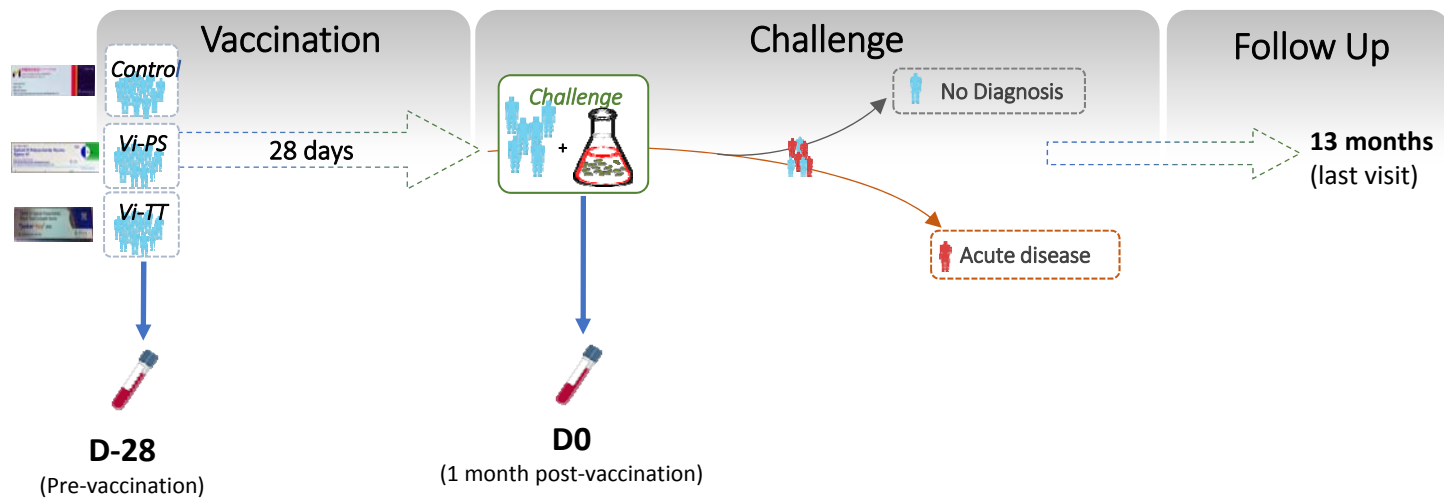
Measurement of antibody dependent neutrophil phagocytosis and the respiratory burst against *Salmonella* Typhi

Mari Johnson – PhD Student



Vaccines Against *Salmonella* Typhi (VAST)

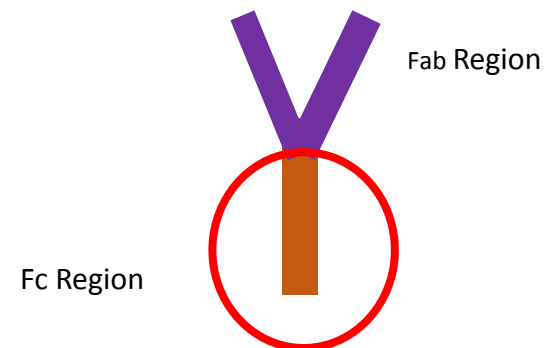
Oxford typhoid fever
Controlled Human Infection Model (CHIM)



***Typhoid Diagnosis* defined as fever $\geq 38^{\circ}\text{C}$ for >12 hours or positive blood culture**

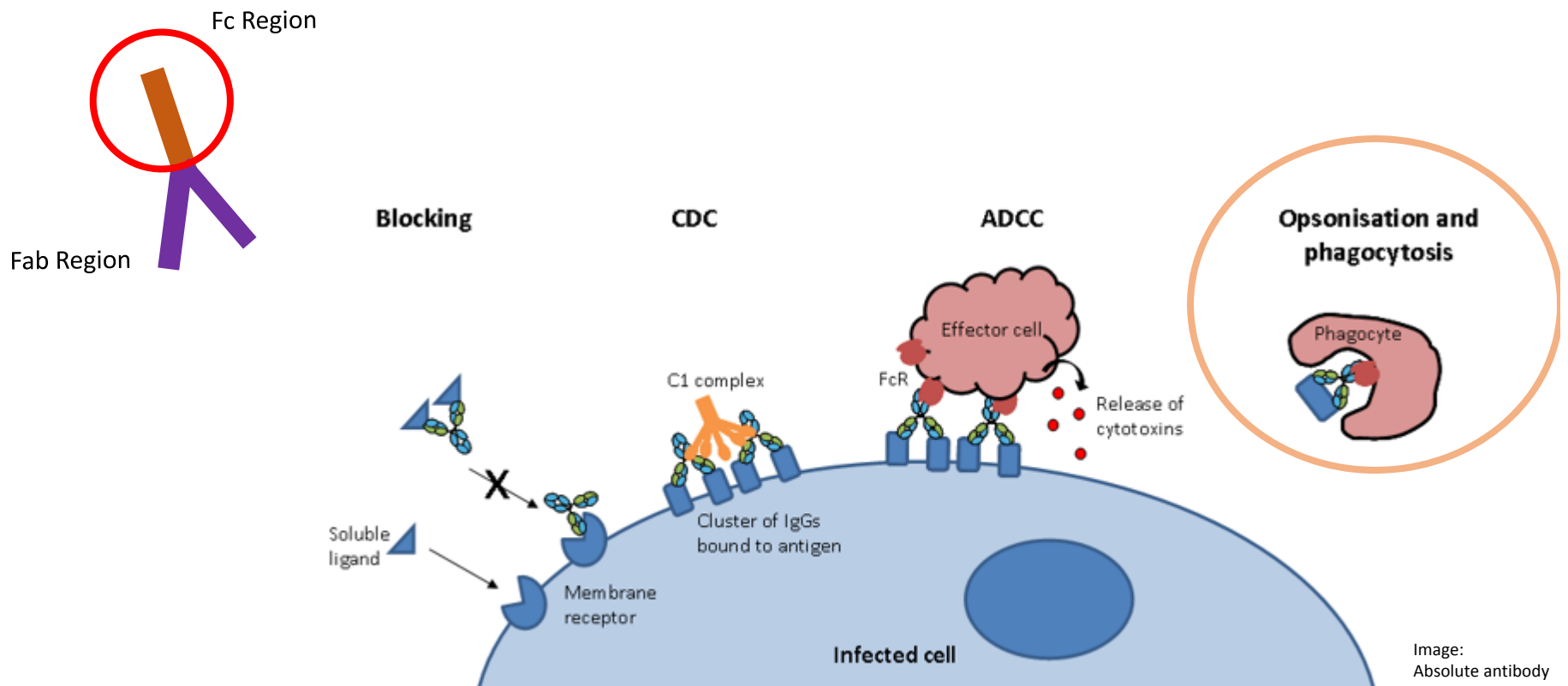
Antibody Response to Typhoid Vaccination

- Found that antibody titre was not significant between diagnosed and non-diagnosed in the Vi-TT group.
- Therefore the type of antibody subclass may be playing a role.
- Antibody subclass is determined by Fc region of the antibody > is responsible for non neutralising functions that help clear the pathogen, including phagocytosis
- These functions could come out as potential correlates of vaccine protection



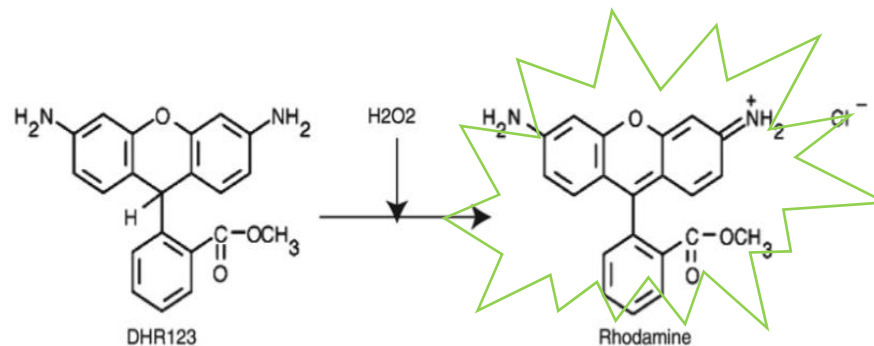
Antibody Dependent Neutrophil Phagocytosis (ADNP)

- Mediated by the Antibody Fc region.
- Fc region can bind to cognate Fc receptors on a variety of effector cells that help induce the inflammatory response and clear the pathogen



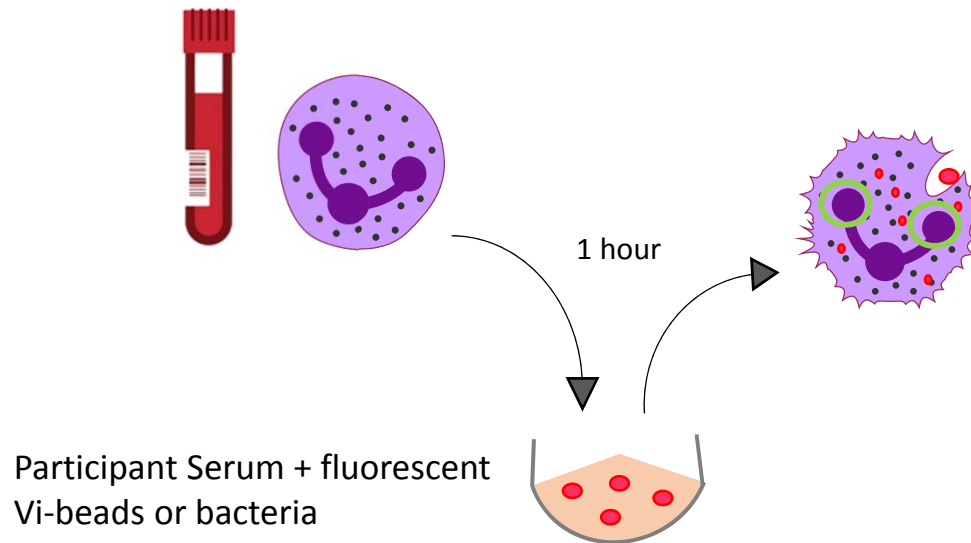
ADNP and the respiratory burst

- Phagocytosis of the bacterium leads to the assembly of NADPH oxidase
- Produces superoxide radicals that can damage bacteria DNA, leading to bacterial cell death
- Respiratory burst can be measured therefore as surrogate marker for bacterial killing
- DHR 123 is a probe that can be oxidised by H_2O_2 into fluorescent form Rhodamine



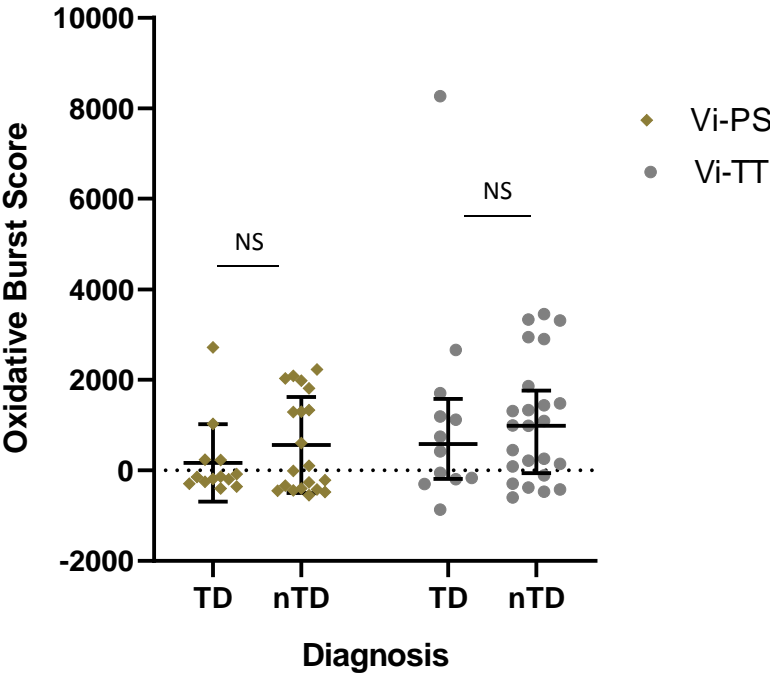
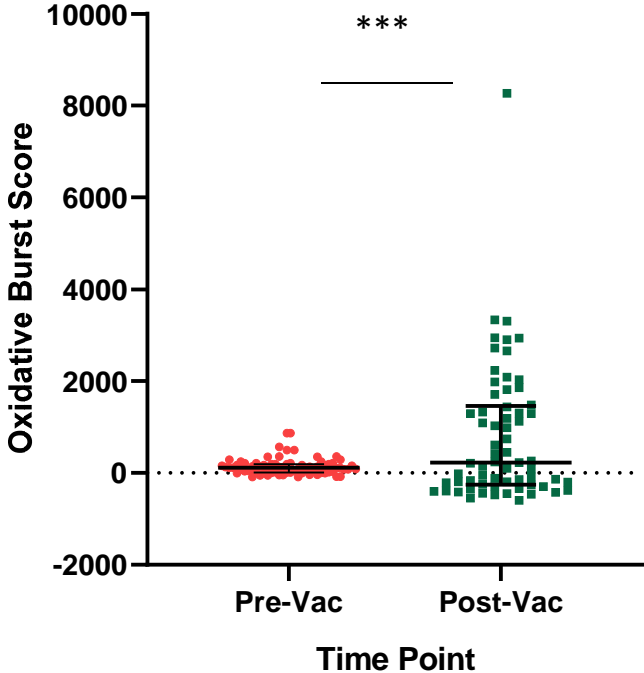
Phagocytosis/Respiratory Burst Assay

- Neutrophils take up antibody coated fluorescent bacteria or beads via phagocytosis and induce the respiratory burst.
- Amount of each fluorescence channel = amount of antibody dependent
Phagocytosis and **Respiratory Burst**



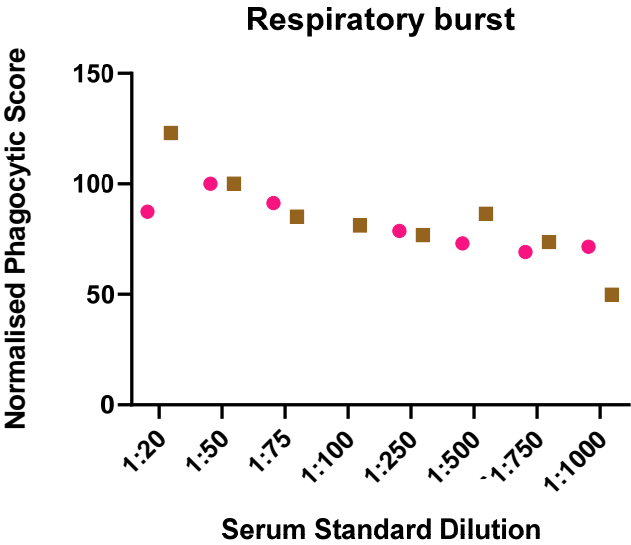
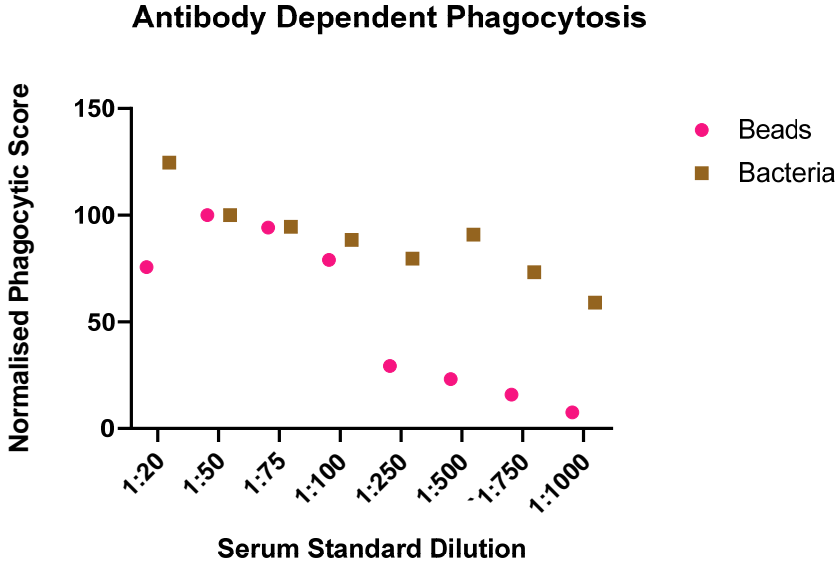
Respiratory Burst - Results:

- Significant increase in respiratory burst seen between pre-vaccination and 28 days post-vaccination
- Increase seen in respiratory burst scores within those that were protected from Typhoid (nTD) in both vaccine arms, however NS.



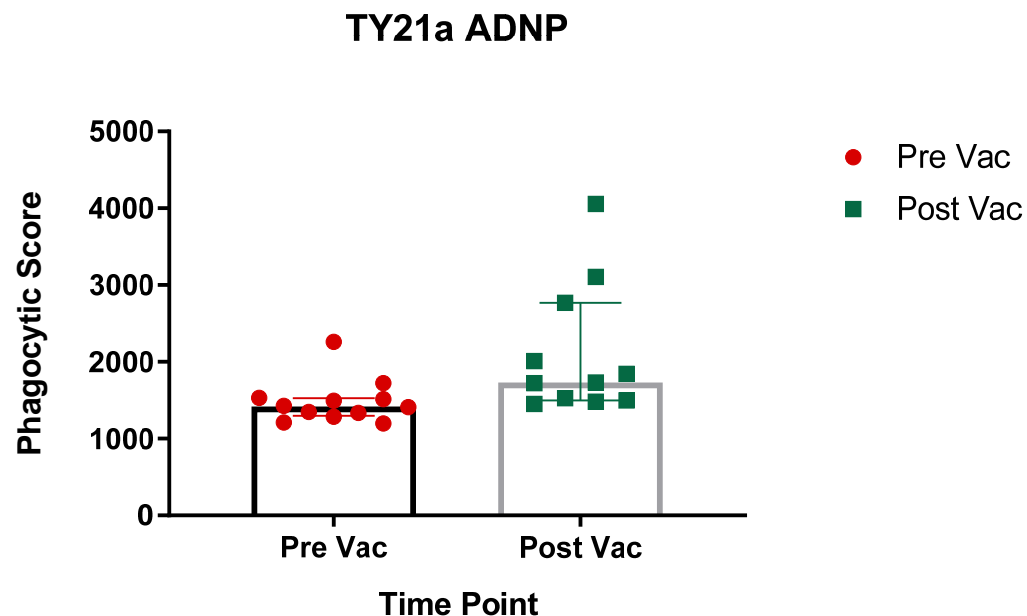
Bacterial Assay

- Aim: Compare bacteria versus previous bead based methods to provide a better representation of *in Vivo* conditions
- To measure antibody dependent phagocytosis and the respiratory burst stimulated with *Salmonella* Typhi



Ty21a phagocytosis assay

- Ty21a Live attenuated strain that does not express Vi.
- Still see an increase in antibody dependent phagocytosis post vaccination with either Vi-PS or Vi-TT vaccines (P=0.05)



Conclusions:

- Antibody dependent neutrophil phagocytosis and the respiratory burst are measures of antibody function that increase post vaccination and may have a role in protection.
- Bacterial assays can show the useful contribution of other antigens that may also be involved in the vaccine response to *Salmonella* Typhi.



Acknowledgements



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