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

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Vaccines Against *Salmonella* Typhi (VAST)

Oxford typhoid fever
Controlled Human Infection Model (CHIM)

**Vaccination**
- Control
- Vi-PS
- Vi-TT

**Challenge**
- No Diagnosis
- Acute disease

**Follow Up**
- 13 months (last visit)

**Typhoid Diagnosis** defined as fever ≥38°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 \( \text{H}_2\text{O}_2 \) 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

Participant Serum + fluorescent Vi-beads or bacteria

1 hour
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**

![Antibody Dependent Phagocytosis](image)

![Respiratory burst](image)
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.
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