Novel neutralizing antibody assays for recombinant human hookworm *Na*-GST-1 vaccine

Xi Chen1, Brian Keegan2, Peter J. Hotez3, Jeffrey M. Bethony2,3, Amar J. Rariwal1

1Department of Microbiology, Immunology and Tropical Medicine, The George Washington University, Washington, DC, USA, 2Department of Pediatrics and Molecular Virology and Microbiology, National School of Tropical Medicine, Baylor College of Medicine, Houston, Texas, USA, 3Fundação Oswaldo Cruz, Instituto René Rachou, Belo Horizonte, Minas Gerais, Brazil.

**Abstract**

**Na**-glomeruloides, a human hookworm causes approximately 85% of the global hookworm infections. Hookworm ingest hemoglobin containing erythrocytes. Hemoglobin is further digested to Heme and Glutathione by hookworm's gut enzymes. Containing Heme is a potent enzyme inhibitor and generates toxic reactive oxygen species which is toxic to hookworms. Hookworm's gut enzyme *Na*G-1 (Necator americanus Glutathione S-transferase 5) has been hypothesized to detoxify Heme. *Na*-GST-1 adjuvanted with Alumagel® is a new vaccine which is currently under clinical development. *Na*-GST-1 has two active sites, the ligand binding or Heme detoxication site (H-site) and the catalytic active glutathione binding site (G-site). We have developed in vitro neutralizing antibody assays (NABA) to assess the neutralizing capacity of antibodies against the functional activity of these two active sites. The antibodies used in this assay were purified from serum of BALB/c mice vaccinated with *Na*-GST-1 vaccine. Also, purified anti-Heme (IgG) and anti-Glutathione (IgG) from Brazilian volunteers plasma from the study below. This study consisted of 6 cohort and each cohort containing 6 volunteers. Antibodies were purified from the study D126 plasma of the 6 volunteers from one of the cohort. This cohort was vaccinated thrice with 100 µg *Na*-GST-1 and 80 µg Alumagel® intramuscularly as shown below.

**Methods**

**Study designs for the generation of antibodies**

1. **Balb/c Mouse Model**

Ten BALB/c mice were immunized 50 µg *Na*-GST-1 + 400 µg Alumagel® intraperitoneally using the following vaccine and bleed schedule. Total as well as specific *Na*-GST-1 IgG was purified from the terminal bleed serum as shown below.

2. **Phase 1 Clinical Trial in Endemic Population (Brazil)**

This study consisted of 6 cohort and each cohort containing 6 volunteers. Antibodies were purified from the study D126 plasma of the 6 volunteers from one of the cohort. This cohort was vaccinated thrice with 100 µg *Na*-GST-1 + 80 µg Alumagel® intramuscularly as shown below.

**Results**

Figure 1. Percent inhibition of the enzymatic activity of *Na*-GST-1 by vs Purified Mouse Polyclonal IgG.

Heme was inhibited in the presence of *Na*-GST-1 specific mouse IgG. Figure 2. *Na*-GST-1 reduces iron-release from Heme.

• *Na*-GST-1 specific mouse IgG.
• *Na*-GST-1 specific Human IgG.

**Conclusion**

In vitro assay showed that *Na*-GST-1 can prevent the release of free iron from Heme.

Total *IgG (micrograms) purified from *Na*-GST-1 vaccinated mice showed a dose-dependent neutralizing capacity against the *Na*-GST-1 enzymatic activity and *Na*-GST-1 hematin-detoxification activity.

Anti *Na*-GST-1 specific IgG (nanograms) purified from the mouse model showed a more sensitive (analytic) dose-dependent neutralizing capacity against *Na*-GST-1 enzymatic activity.

Anti *Na*-GST-1 specific IgG purified from Phase I Brazilian Adults showed a dose dependent neutralizing capacity against *Na*-GST-1 hematin-detoxification activity. The hematin-detoxification activity of *Na*G-1 was inhibited by 50% using 5.0 µg of *Na*-GST-1 specific human IgG.

These neutralizing antibody assay results based on neutralizing the Heme protective function of *Na*-GST-1 can potentially generate an immune correlate of protective immunity for the Phase 2 Clinical Trial.

**Acknowledgments**

This project is supported by the Sabin Vaccine Institute through funding from the Bill and Melinda Gates Foundation and the Dutch Government. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this Poster apart from those disclosed.