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

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Abstract

Necator americans, a human hookworm causes approximately 85% of the global hookworm infections. Hookworm ingest hemoglobin containing erythrocytes. Hemoglobin is further digested to Heme and the global hookworm infections. Hookworm ingest hemoglobin which is currently under clinical development.

Methods

NADA based neutralizing the enzymatic activity of Na-GST-1

This assay utilizes CDNB (1-Chloro-2,4-dinitrobenzene) a substrate which evaluates the catalytic activity of Na-GST-1 in 0.1 M Potassium phosphate buffer (pH 6.5) in presence of GSH (glutathione). To determine the neutralizing capacity of antibodies against the enzymatic activity, the purified IgG was incubated with the above components at 37°C for 60 min. The experimental steps for the assay are shown below.

Enzymatic Activity

GSH + CDNB Substrate – Na-GST-1 (GS-DNB Conjugate + HCl)

Neutralization of Enzymatic Activity

GSH + CDNB Substrate + Na-GST-1 (GS-DNB Conjugate + HCl)

The rate of increase in the absorbance at 340nm is directly proportional to the GST Activity.

Results

Figure 2. Na-GST-1 reduces iron-release from Hematin

100 µM Hematin, 100 µM Prototypophorphyrin IX, 100 µg guanacaste M, 100 µg ⁎GST-1 and 50 µg Na-GST-1 was used in vivo assays. Iron detection reagent contains 0.244M ferrozine,0.1M ammonium acetate and 1M acetic acid in water (pH 7.4).

Figure 2 Panel A, shows the hematin protective function of Na-GST-1. This study utilized Nematode (H. americanus) N. americanus, ligand binding or Heme detoxification site (H-site) and the global hookworm infections. Hookworm ingest hemoglobin which is currently under clinical development.

Figure 2 Panel B, shows that the Na-GST-1 function is specific to hematin and does not have any effect on any other iron containing molecule. A Mann–Whitney U test was used to statistically compare the groups with a significance level α = 0.05.

Figure 3. Neutralization of Hematin detoxification activity of Na-GST-1 by mouse polyclonal IgG

Na-GST-1 (Iron releasing assay) based on neutralizing the Hematin protective function of Na-GST-1 (Iron releasing assay). This study utilizes Hematin (H2O2 oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (Iron detection reagent). This assay estimates the Na-GST-1 activity by measuring the iron release from the Hematin using ferrozine reagent (iron detection reagent).

Figure 4, shows the iron released from 250 µM Hematin when incubated with 5 µg Na-GST-1 and 100 mM H2O2 and IgG at 37°C.

Figure 5. Neutralization of Hematin detoxification activity of Na-GST-1 by Anti Na-GST-1 specific Human IgG

This assay utilizes Hematin, H2O2 (oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (iron detection reagent). This assay utilizes hematin, H2O2 (oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (iron detection reagent). This assay utilizes hematin, H2O2 (oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (iron detection reagent). This assay utilizes hematin, H2O2 (oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (iron detection reagent). This assay utilizes hematin, H2O2 (oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (iron detection reagent). This assay utilizes hematin, H2O2 (oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (iron detection reagent). This assay utilizes hematin, H2O2 (oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (iron detection reagent). This assay utilizes hematin, H2O2 (oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (iron detection reagent). This assay utilizes hematin, H2O2 (oxidizer), Trichloroacetic acid (TCA), Ascorbic Acid (reducing reagent) and Ferrozine (iron detection reagent).

Results

Figure 5. Neutralization of Hematin detoxification activity of Na-GST-1 by Anti Na-GST-1 specific Human IgG

• Total Polyclonal IgG from Commercial Plasma and Non-Endemic (NE) human Plasma.
• Anti Na-GST-1 specific Human IgG.

Figure 6. Percent inhibition* at five different levels of Na-GST-1 specific Human IgG.

• DCl of Anti Na-GST-1 specific Human IgG with the remaining components compared to the absence of Na-GST-1 specific Human IgG.
• Anti Na-GST-1 specific IgG (nanograms) purified from the mouse model
• Anti Na-GST-1 specific Human IgG.

Conclusion

• In vitro assay showed that Na-GST-1 can prevent the release of free iron from Hematin.
• 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 (analyzer) dose-dependent neutralizing capacity against Na-GST-1 hematin-detoxification activity.
• Anti Na-GST-1 specific IgG purified from Phase 1 Brazilian Adults showed a dose dependent neutralizing capacity against Na-GST-1 hematin-detoxification activity. The hematin-detoxification activity of IgG of Na-GST-1 was inhibited by 50% using 5 µg of Na-GST-1 specific human IgG.
• These neutralizing antibody assay results based on neutralizing the Hematin protective function of Na-GST-1 can eventually generate an immune correlates of protection (ICP) analyses from the Phase 2 Clinical Trial.

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