Thermostable Subunit Vaccine Results in Protective Immunity in Rhesus Macaques in an Inhalational Ricin Model

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ABSTRACT

Ricin is a plant-derived toxin that, when inhaled in sufficient quantity, causes a rapidly progressive respiratory syndrome that can result in death. A vaccine derived from the A-chain moiety (RiVax™) has been developed by using conventional aluminum adjuvant, lyophilized in conjunction with glassifying excipients, and tested in a nonhuman primate (NHP) model of inhalational ricin toxicity. Rhesus macaques were either sham-vaccinated or vaccinated three times one month apart with 100 micrograms of lyophilized vaccine reconstituted with Water for Injection. NHPs were exposed to an aerosolized ricin toxin (~3 x LD50) 45 days after the last vaccination. In the first of two cohorts of six NHPs each, all of the NHPs in the vaccinated group survived exposure (6/6), with no adverse signs of gross lung pathology. In the second cohort, 5/6 vaccinated NHPs survived; one NHP was euthanized on the 7th day post-exposure. Because a delayed death is atypical in this model, it is hypothesized that death was due to a secondary bacterial infection not related to ricin exposure; pathology is pending. All NHPs in both control groups (6/6) died within ~36 hours of exposure and developed severe lung damage, including characteristic hemorrhage and edema. All of the vaccinated NHPs developed ELISA-reactive antibodies after two vaccinations and toxin neutralizing antibodies after the third vaccination. Previous studies with this lyophilization technology have demonstrated that the RiVax™ vaccine is stable for at least 12 months at 40°C and the retained potency is associated, in part, with retention of the native configuration of the antigenic portion of the protein. Thus, this prototype vaccine overcomes cold-chain requirements. It has demonstrated safety and efficacy in a lethal aerosol NHP model of ricin toxicity. The results of this study indicate that RiVax™ has great promise for protection against ricin exposure in humans and further clinical development of RiVax™ is ongoing to establish its safety profile.

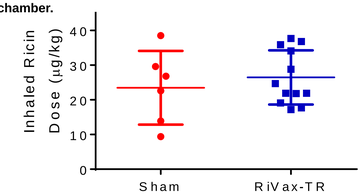
Acknowledgements: This research was supported with funding from NIH/NIAID grants U01 AI08 2210, A1-070236 and in part by NIH-OD-011104-53 (Tulane National Primate Center Base Grant). These results have been recently published in Roy et al. 2015.

STUDY DESIGN

Male and female Rhesus macaques were vaccinated on Days 0, 30 and 60.

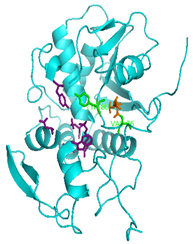
Group	N	Dose Regimen (IM)	ELISA & TNA Timepoints
Control	6	Sham vaccinated	Days 1 (ELISA only), 14, 30, 45, 60, 75, 110
RiVax™-TR	12	100 μg RiVax™-TR	

Aerosol exposure: After plethysmography measurements, each anesthetized monkey was challenged with ricin aerosol on Day 110 using the head-only aerosol exposure system (performance characteristics established previously). This challenge (target 3xLD_{50}) was performed in a class III biosafety cabinet within the BSL-3 level laboratory. Ricin aerosols were generated directly into the chamber using a Collison three-jet nebulizer (BGI Inc., Waltham, MA). Integrated air samples were obtained during exposure using an all-glass impinger (AGI) drawing from a port centered on the side of the chamber.



BACKGROUND

Ricin, a plant toxin capable of being weaponized, has well documented toxicity. It is known to be lethal by the aerosol route, resulting in epithelial necrosis within hours of exposure, multifocal hemorrhagic edema and death within 12-36 hours. RiVax™-TR contains a modified ricin A-chain (RAC), genetically altered to eliminate both the toxicity attributed to the enzymatic activity of ricin (active site modification) as well as the toxicity attributed to vascular leak, which is a secondary toxicity [vascular leak site (VLS) modification].



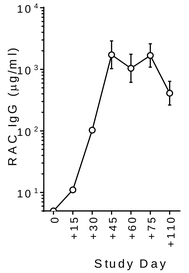
Ribbon diagram of RiVax™ (Protein Data Base, PDB,1RTC):

- Active site in purple
- VLS site in orange
- Residues mutated (Y80, V76) to inactivate each site in green
- Active site residue Y80 was changed to A and the VLS V76 residue was changed to M

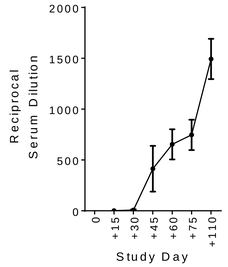
RESULTS

Immune response to RiVax™-TR: RAC-specific IgG & Neutralizing Antibodies

Anti-RAC



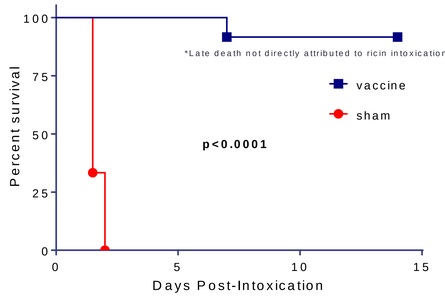
Neutralizing Ab



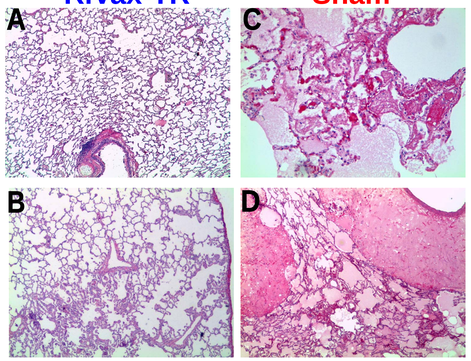
- Neutralizing antibodies believed to correlate with protective efficacy.
- Neutralizing antibodies consistently observed after 3 vaccinations (minimum level 640).
- Neutralizing antibody response on Day 110 measured prior to ricin exposure, suggesting that the generation of neutralizing antibodies is relatively slow.

RESULTS

Survival: RiVax™-TR against Aerosolized Ricin



Significantly Less Lung Damage in RiVax™-TR Vaccinated Animals by Histopathology

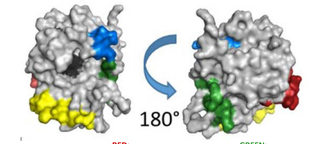


Histopathology of lungs from animals either vaccinated with RiVax™-TR (Panels A, B) or sham vaccinated (Panels C, D) and then exposed to a lethal dose of aerosolized ricin (Roy et al. 2015):

- Panel A (RiVax™-TR): otherwise unremarkable normal lung;
- Panel B (RiVax™-TR): mild hyperplasia and focal inflammation;
- Panel C (Sham): marked edema and lung fibrin accumulation; and
- Panel D (Sham): massive edema and associated inflammation.

RESULTS

Epitope Profile in NHPs Similar to Humans



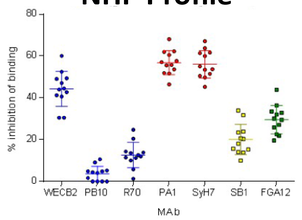
BLUE:
WECB2 (non-linear, neutralizing)
PB10 (linear, neutralizing)
R70 (linear, neutralizing)

RED:
PA1 (non-linear, neutralizing)
SyH7 (linear, neutralizing)

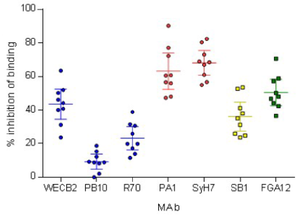
GREEN:
FGA12 (linear, non-neutralizing)

YELLOW:
SB1 (linear, non-neutralizing)

NHP Profile



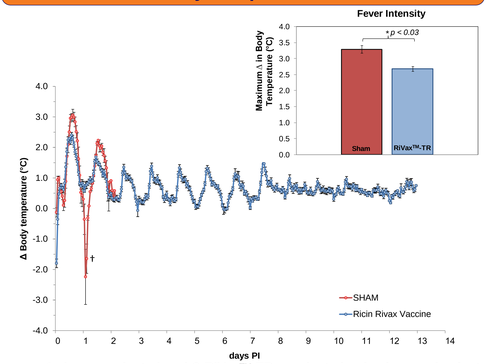
Human Profile



- Mouse monoclonal antibodies, recognizing defined epitopes, were used in a competitive ELISA with NHP or Human sera to determine the epitope profile.
- Human samples obtained from a Phase 1 clinical study with RiVax formulated as a liquid alum suspension.
- For both the human and NHP profiles, WECCB2 vs PB10/R70 and PA1/SyH7 vs PB10/R70 are statistically significantly different (p<0.,001)
- In mice, a linear epitope (aa 97-108) defined by Mabs PB10/R70 is immunodominant.

RESULTS

Ricin Intoxication Associated with Changes in Body Temperature



- 4 sham-vaccinated and 8 RiVax™-TR vaccinated animals were implanted with telemetry devices 30 days prior to initiating vaccination (Day -30).
- Telemetry data was monitored throughout vaccination (not shown) and after ricin intoxication (see above graph).
- Statistically significant differences in body temperature fluctuations and heart rate (not shown) were observed after ricin intoxication between the Sham-vaccinated and RiVax™-TR vaccinated animals.
- No changes in telemetry were observed during vaccinations with either the Sham vaccine or the RiVax™-TR vaccine (data not shown).

CONCLUSIONS

- RiVax™-TR was 100% (11/11) efficacious in the NHP primate model of lethal aerosolized ricin toxin.
- RAC antibodies were significantly increased after 2 vaccinations while neutralizing antibodies were consistently elevated after 3 vaccinations.
- The epitope profile of the neutralizing antibody response was similar in NHPs and humans.

References

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