Poster #215

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

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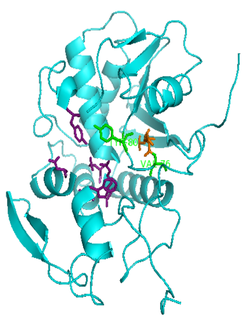
ABSTRACT

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 LD_{50}) 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 (immunoglobulin G (IgG)) 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. This research was supported with funding from NIH/NIAID grant U01 AI08 2210.

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. Antibodies to the ricin A chain (RAC) toxin can prevent morbidity and mortality, therefore a vaccine is considered the most feasible means to address the possibility of a biological attack of aerosolized ricin. The vaccine component that has been tested in this study is RiVax™-TR. RiVax™-TR contains a modified 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]. The drug product is lyophilized as an aluminum-adsorbed product for reconstitution with sterile Water for Injection, USP.



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.

STUDY DESIGN

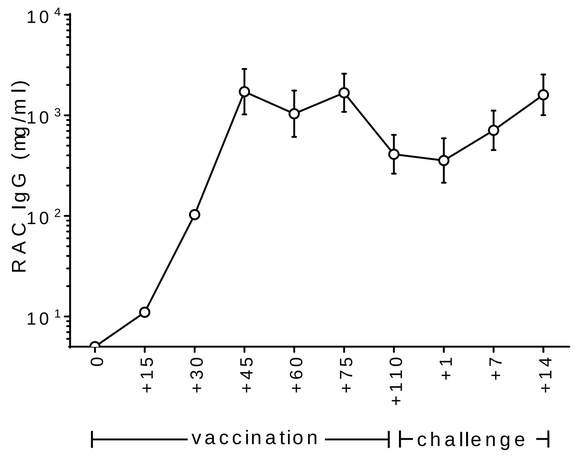
Male and female Rhesus macaques were vaccinated a total of three times, on Days 0, 30 and 60. On Day 110, NHPs were aerosol exposed to a target dose of 3xLD_{50} of ricin toxin. Clinical observations were recorded at least twice daily. Survivors were humanely euthanized 14 days post-challenge and gross necropsies were performed on all NHPs.

Group	N	Dose Regimen (IM)	ELISA Timepoints	TNA Timepoints
Control	6	Sham vaccinated	Days 1, 14, 30, 45, 60, 75	Days 15, 30, 45, 60, 75
RiVax™-TR	12	100 mg RiVax™-TR		

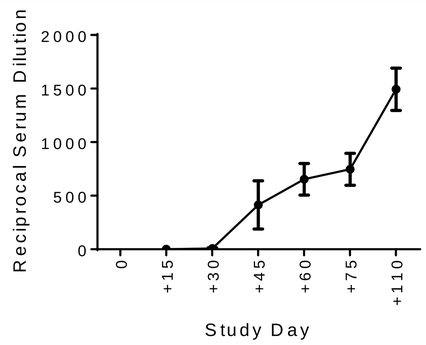
Aerosol exposure: After plethysmography measurements, each anesthetized monkey was challenged with ricin aerosol using the head-only aerosol exposure system (performance characteristics established previously). This challenge was performed in a class III biosafety cabinet within the BSL-3 level laboratory. 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.

RESULTS

Immune response to RiVax™-TR: RAC-specific IgG

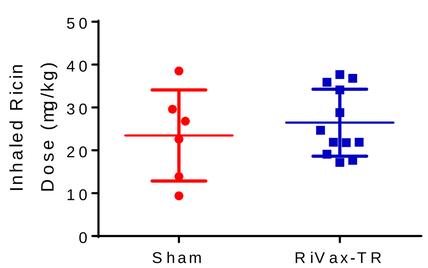


Immune response to RiVax™-TR: Neutralizing Antibodies

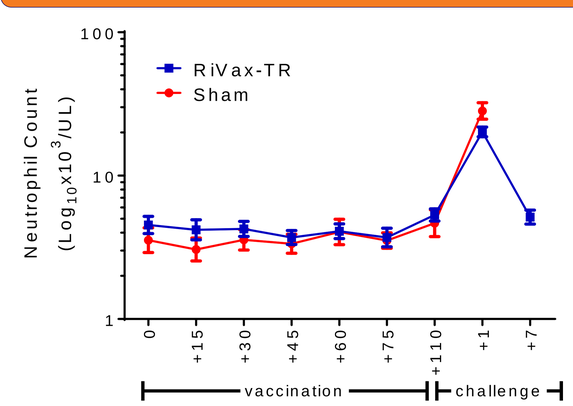


- Neutralizing antibodies believed to correlate with protective efficacy.
- Neutralizing antibodies consistently observed after 3 vaccinations (minimum level 640).
- Neutralizing antibody response on Day 110 measured prior to ricin exposure, suggesting that the generation of neutralizing antibodies is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

Ricin aerosol challenge: Comparable Among Groups

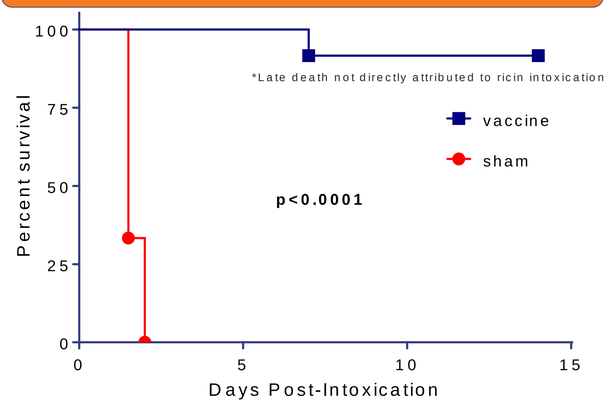


Neutrophilia Observed after Ricin Intoxication in both Sham and RiVax™-TR Vaccinated Animals

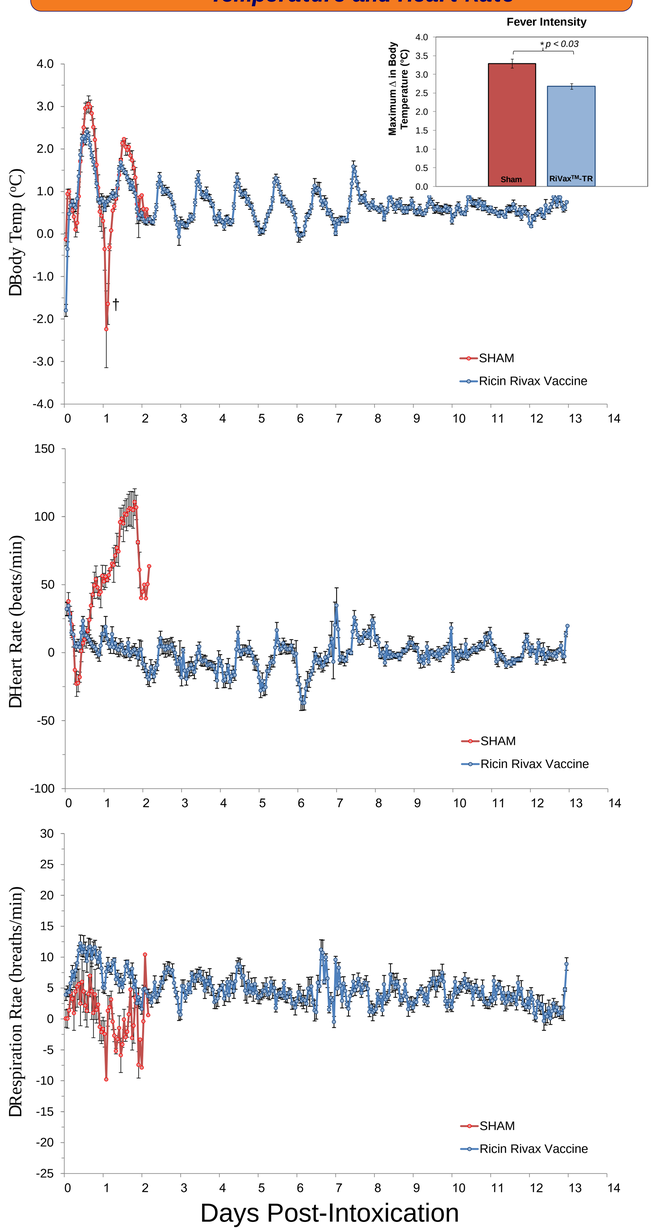


RESULTS

Survival: RiVax™-TR against Aerosolized Ricin



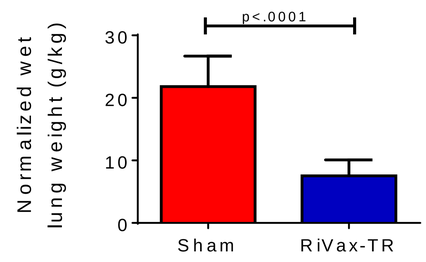
Ricin Intoxication Associated with Changes in Body Temperature and Heart Rate



- 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 graphs)
- Statistically significant differences in body temperature fluctuations and heart rate 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).

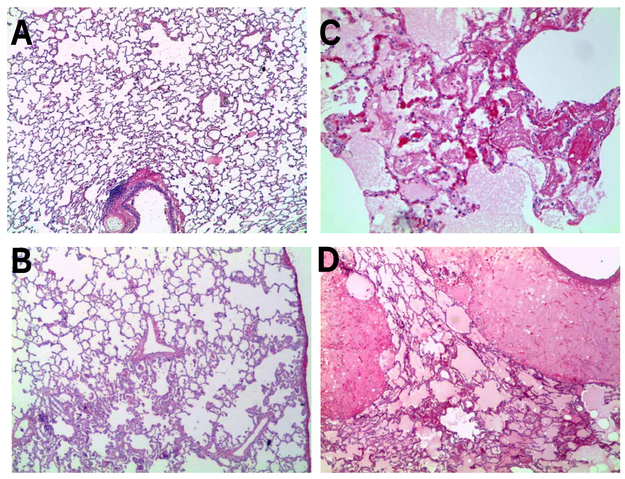
RESULTS

Lungs Significantly Enlarged at Necropsy after Ricin Intoxication in Sham-vaccinated Animals



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

RiVax-TR Sham



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:

- 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.

CONCLUSIONS

- RiVax™-TR was 100% (11/11) efficacious in the NHP primate model of lethal aerosolized ricin toxin.
- Animals were exposed to an aerosol of ricin toxin 3-5 times the amount that is known to result in death of untreated animals.
- RAC antibodies were significantly increased after 2 vaccinations while neutralizing antibodies were consistently elevated after 3 vaccinations.
- Heart rate and temperature were significantly different in RiVax™-TR vs. Sham vaccinated individuals.
- Morbidity was also improved, with significant differences in both lung organ weights and lung histopathology on necropsy.
- Neutrophilia occurred in both RiVax™-TR and Sham-vaccinated animals, indicating a robust innate immune response to ricin intoxication.

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