Using Monoclonal Antibodies as Immune Correlates of Protection: Thermostable Ricin Toxin Vaccine Development

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ABSTRACT

Ricin is a plant-derived toxin that causes a rapidly progressive respiratory syndrome that can result in death when inhaled even in small amounts. Ricin toxin is easily derived from castor bean production and constitutes a serious biological threat agent. Soligenix is developing a ricin-toxin vaccine derived from the A-chain moiety of ricin (RiVax®), adjuvanted with aluminum hydroxide and thermostabilized via lyophilization in conjunction with glassifying excipients. RiVax has demonstrated 100% protection in a rhesus macaque model of aerosolized ricin exposure (1) and safety in two phase 1 clinical studies (2). Development of a ricin toxin vaccine will require use of the "Animal Rule" (21 CFR 601.90-601.95). Use of the Animal Rule dictates the identification of immune correlates of protection that can be translated between human studies and animal models. Recent studies have evaluated immunogenicity measures, including total anti-ricin IgG, neutralizing antibody levels, and epitope specific antibody competition profiles as potential immune correlates of protection. Epitope competition assays have been specifically developed using neutralizing monoclonal antibodies with known recognition sites (epitopes) on ricin/RiVax. Studies in mice, non-human primates, and humans have suggested that the epitope competition profiles in particular are similar across species (1) and, moreover, that threshold levels of epitope competition may be predictive of survival to subsequent ricin challenge in mice and non-human primates. The stability of these same epitopes on the RiVax protein are also predictive of RiVax drug product potency. In aggregate, these results suggest that epitope recognition may be a powerful tool for vaccine development, particularly under the Animal Rule. Approval of a ricin toxin vaccine will result in an increased ability to protect both the warfighter and the emergency responder civilian populations from an easily produced and dispersed biological threat agent.

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METHODS

Potency testing of different formulations of RiVax routinely involves both *in vivo* vaccination-challenge studies to confirm efficacy in both mice and non-human primates (rhesus macaques) and *in vitro* binding competition assays. A key aspect of these studies is the correlation between immunogenicity assays conducted with blood from vaccinated individuals collected prior to challenge and individual survival post-challenge. Immunogenicity measures include total anti-ricin IgG endpoint titers and a newly developed "EPICC" assay. Total neutralizing antibodies were not routinely evaluated due to low assay sensitivity.

Key to the *in vitro* and *in vivo* evaluations was the use of mouse monoclonal antibodies with known epitope binding to ricin toxin A-chain (RTA) and RiVax. Of particular utility were the PB10 and SyH7 antibodies binding to Cluster 1 and 2 of RTA/RiVax, respectively (5). Both antibodies recognize linear epitopes and are known to be toxin neutralizing both in vitro and in vivo (5).

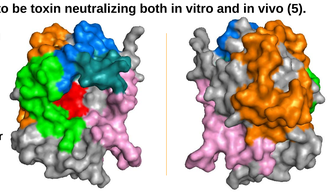
Antigenic clusters on RTA (front and back).

Cluster 1: PB10, WECB2, R70 (blue/teal);

Cluster 2 (orange) SyH7, PA1, PH12, TB12;

Cluster 3 (green) IB2; Cluster 4 (pink) GD12, JD4;

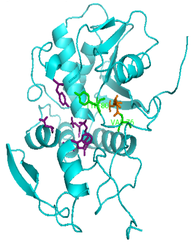
Active site (red):



METHODS

RiVax®

RiVax contains a modified RTA, genetically altered to eliminate both the toxicity attributed to the enzymatic activity of ricin (active site modification) as well as the secondary toxicity attributed to vascular leakage [vascular leak syndrome (VLS)].



Ribbon diagram of RiVax (Protein Data Base, PDB1RTC):

- Active site in purple
- VLS site in orange
- Residues mutated (Y80A, V76M) to inactivate each site in green

EPICC Assay

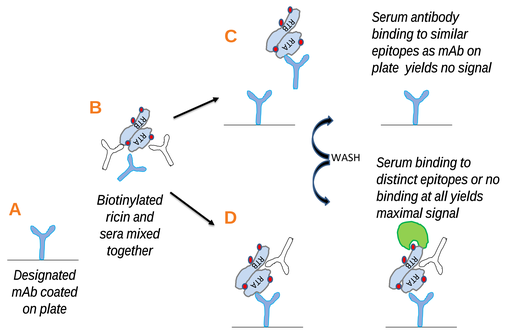
- *Epitope Profiling Immuno-Capture Competition* (EPICC) ELISA assay:

A. Coat microtiter plates with capture mAb (1 µg/ml);

B. Incubate pre-determined EC90 concentration of biotinylated ricin (BR) with competitor mAb/mAb mixture or polyclonal antibody (pAb) serum;

C. Apply BR/m(p)Ab mixture to mAb-coated wells. A reduction of BR bound to the capture mAb will result from the presence of competitor mAbs that recognize the same or overlapping epitopes; bound BR is detected using streptavidin-HRP;

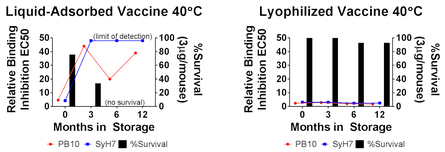
D. Alternatively, BR will be captured with high efficiency in the absence of competitor antibodies



RESULTS

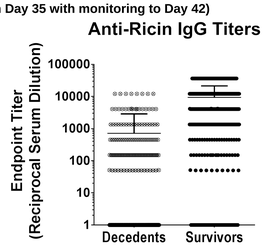
In Vitro Binding and Protective Efficacy in Mice

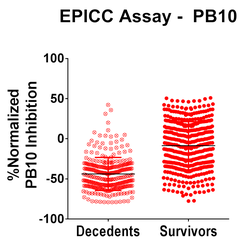
- Relative in vitro EC_{50} binding competition between RTA and RiVax recorded as a function of RiVax storage time and temperature revealed the stability of the thermostabilized vaccine and the instability of the liquid-adsorbed vaccine formulation when evaluated with the SyH7 or PB10 monoclonal antibodies
- In vivo vaccination challenge studies in mice further revealed a complete loss of protective efficacy in the liquid adsorbed formulation stored at 40°C at the 6 month timepoint.

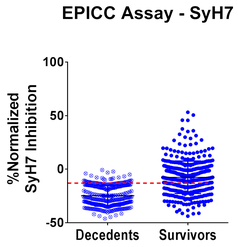


Immunogenicity Correlates in Mice

- Immunogenicity and survival data was compiled from 4 individual studies (689 female BALB/c mice) evaluating efficacy/potency of various RiVax formulations using the same experimental design (i.e., vaccinations on Days 0 and 21, blood draws on Day 30 and intraperitoneal challenge with 5xLD_{50} ricin on Day 35 with monitoring to Day 42)





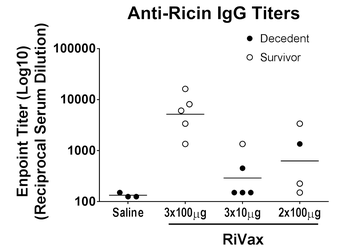


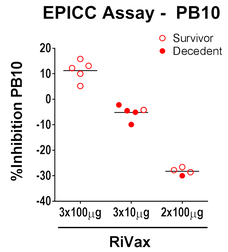
RESULTS

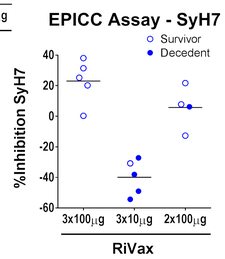
Protective Efficacy in Non-Human Primates

- Vaccinations (lyophilized formulation) were administered on Days 0, 30 and 60 with blood collection on Day 105 and aerosolized ricin challenge (3xLD_{50}) on Day 110 with survival monitored to Day 124 (4).

Group	N (M/F)	Dose (µg protein)	Vaccination Schedule	%Survival (3xLD_{50} aerosol)
Saline	1/2	0	Days 0, 30, 60	0
RiVax	2/3	100		100
RiVax	2/3	10		20
RiVax	2/2	100	Days 0, 30	75







RESULTS

Predictive Accuracy of the EPICC SyH7 Assay

- Using a training set of 143 mice, a binary classification model was derived and applied to the prospective test set (remaining 546 mice), yielding an overall accuracy of 74%.

Actual Outcome	Predicted Outcome		Total	%Accuracy (Predicted /Total)
	Survivors	Decedents		
Survivors	212	104	316	67
Decedents	36	194	230	84
Overall (Survivors + Decedents)			546	74

- Preliminary model (not yet fully optimized) predicts decedents very accurately, facilitating clinical use as an indicator of individuals needing an additional "booster" shot.

CONCLUSIONS

- Previous studies demonstrated stability and potency of lyophilized thermostabilized formulations of RiVax, consistent with results shown here.
- Endpoint titers have limited correlative ability to predict survival, while specific competition assays with monoclonal antibodies yield significantly more sensitivity.
- Enhanced sensitivity of the SyH7 antibody in both in vitro and in vivo assays suggests the importance of the "cluster 2" domain of RiVax to protective efficacy.
- Qualitative consistency of response across species suggests that immunogenicity endpoints, particularly those utilizing the monoclonal antibody competition responses, may be used to demonstrate feasibility of animal models to mimic the human condition, as required by the Animal Rule.
- Quantitative consistency of immunogenicity response will enable definition of a human equivalent dose upon completion of planned Phase 1/2 clinical study with the thermostabilized formulation.

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