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TITLE: THE DEVELOPMENT OF HUMAN MONOCLONAL ANTIBODIES AGAINST
RICIN BY IN VITRO STIMULATION (SBIR 90.I)

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This SBIR proposal describes the development of an approach to immuno-prophylaxis against ricin toxin (RCA 60). We plan to produce a ricin toxoid vaccine for the development of murine and human monoclonal antibodies against ricin toxin. In Phase I, we propose to produce and characterize a candidate ricin toxoid vaccine and use this vaccine for in vivo stimulation of murine lymphocytes and in vitro stimulation of human lymphocytes that secrete antibodies against ricin toxin. In Phase II, murine and human hybridomas secreting neutralizing monoclonal antibodies against ricin toxin would be produced by fusing Ag. 653 to muring splenocytes or EBV-transformed human lymphocytes to the heteromyeloma fusion partner SHM-D33. Neutralizing monoclonal antibodies are expected to be useful for both the prophylaxis and therapy of ricin poisoning.

RA I; BD; SBIR Phase I; Monoclonal; Ricin; Vaccine

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Section I. Introduction.

This SBIR proposal describes the development of an approach to immunoprophylaxis against ricin toxin (RCA 60). We plan to produce a ricin toxoid vaccine for the development of murine and human monoclonal antibodies against ricin toxin. In Phase I, we propose to produce and characterize a candidate ricin toxoid vaccine and use this vaccine for in vivo stimulation of murine lymphocytes and in vitro stimulation of human lymphocytes that secrete antibodies against ricin toxin. In Phase II, murine and human hybridomas secreting neutralizing monoclonal antibodies against ricin toxin would be produced by fusing Ag. 653 to murine splenocytes or EBV-transformed human lymphocytes to the heteromyeloma fusion partner SHM-D33. Neutralizing monoclonal antibodies are expected to be useful for both the prophylaxis and therapy of ricin poisoning.

This Phase 1 proposal has three major objectives:

Objective 1. Development and characterization of a detoxified ricin vaccine.

Objective 2. Demonstration of a protective antibody response in vaccinated animals with aforementioned vaccine.

Objective 3. In vitro stimulation of human lymphocytes with ricin-toxoid vaccine.



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Section II. Progress to Date.

1. Characterization of commercially available ricin.

i. Whole ricin (RCA 60) was obtained commercially for production of vaccine. This toxin was handled in accordance with SOP's that exist in our laboratories for highly toxic substances. All work involving potential exposure to the native toxin was conducted by highly experienced personnel working with the toxin in a biological safety cabinet.

2. Production of toxoid vaccine against ricin toxin (RCA 60).

i. Formaldehyde inactivation: Whole ricin toxoid was prepared by formalin inactivation. Toxin was incubated with formaldehyde for 3 days; stationary phase, surface to air evaporation to precipitate vaccine was performed.

ii. Murine toxicity testing: Vaccine was non-toxic at the highest dose tested (25 ugs. per mouse) in 6 mice. This represents 50 LD₁₀₀s in our model. Vaccine and the procedure for its production were provided to the contractor as required in SBIR.

3. In vitro ricin quantitative/neutralization assays.

i. A human hybridoma cell line (12-11) secreting antibody to lipid A, owned by Univac Biologics and a CHO cell assay have been used to develop sensitive tissue culture assays for quantitation of ricin toxin and neutralization.

ii. This assay detects toxin concentrations down to at least 15 picograms/ml. These assays were modified to detect neutralizing antibody to ricin toxin.

iii. Rabbit antisera to ricin toxoid were prepared which neutralize native toxin in the above assays.

4. Production of in vitro stimulated EB virus transformed human lymphocytes secreting antibody to toxoid vaccine against RCA60.

i. Attempts were made to perform in vitro stimulation with our inactivated ricin vaccine and commercial available A or B chain. Both vaccine and subunits of ricin agglutinated lymphocytes and monocytes in our preparations preventing successful preparation of antigen specific antibody secreting EBV transformed lymphocytes.

ii. Ricin western blot strips were sent to a collaborator at Stanford University, Dr. Michele Glasky, working in Dr. Nelson Teng's laboratory. This laboratory possesses a library of human monoclonal antibodies. The tenth human clone screened contained antibody to ricin by western blot. A copy of Dr. Glasky's letter and the western blot results were provided to Major Paul Lemley in December, 1990.

5. Production of neutralizing murine monoclonal antibodies to toxoid vaccine against ricin toxin (RCA 60). (The phase I SBIR expired in September 1990. This was work proposed in a phase II SBIR and has been completed between August 1990 and February 1991.)

i. Two of six vaccinated, antibody positive mice have been challenged with 500 ugs. of ricin toxin. No deaths occurred in 72 hours. These animals have been splenectomized and their lymphocytes fused to Ag. 653 using PEG. Hybridomas #70 and #138, secrete antibody to A chain of ricin by western blot. These hybridomas were doubly cloned, stabilized and frozen.

ii. Murine monoclonal antibodies (MAB) #70 and #138 neutralized ricin toxin (RCA 60) in our in vitro neutralization assay.

iii. Murine monoclonal antibodies (MAB) #70 and #138 protected against 2 LD₁₀₀s of ricin toxin in our murine protection model.

iv. Rabbit antisera, hybridoma supernatants, ascites and column purified antibody were provided to Major Paul Lemley in January 1991 for testing in his in vitro and in vivo animal systems.

Section IV. Work that could be performed in a phase II SBIR by Univax Biologics.

i. GMP grade ricin (RCA 60) toxoid vaccine could be produced by Univax Biologics for active immunization of soldiers.

ii. GMP grade murine monoclonal antibodies (#70 and 138) could be produced by Univax Biologics for passive treatment or prophylaxis of soldiers.

iii. Monoclonal antibodies #70 and #138 could be humanized by Univax Biologics.

iv. Additional human clones could be identified by screening hybridoma libraries for antibody to ricin toxin (RCA 60) and tested for neutralizing activity in vitro and in vivo systems.

Section V. Administrative Comments.

None.