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14. ABSTRACT: Ricin, a type II ribosome inactivating protein (a heterodimeric glycoprotein containing two subunits joined together by a disulfide bond), is a biological toxin with a history of use as a weapon of war and bioterrorism. Biological toxins act in concert with various (mostly unknown) host proteins; specific host proteins are essential for toxin action. In an effort to identify possible targets for pharmaceutical intervention that may lead to an effective treatment for ricin toxicosis, this study investigates the effects of inactivating specific host proteins and cellular response when exposed to ricin. Specifically, 278 genes were selected for challenge based on the criteria developed by Lexicon Genetics to identify genes that might encode pharmaceutically tractable proteins. A full phenotypic analysis of all knockout mouse lines (278) was performed, and fibroblast cell cultures were established for all 278 KO lines. A kill curve was established for the mouse fibroblast cells, and the fibroblast cell cultures were challenged in triplicate (3 separate homozygous knockout mice for each KO line) with ricin at different points in the curve. Through a cooperative research and development agreement (CRDA) with investigators at USAMRIID, specific whole animal gene knockout models will also be made available for testing of modified responses to ricin toxin; this task awaits approval from the USAMRMC Animal Care and Use Review Office (ACURO). A 12-month extension of the performance period (to December 14, 2006) was granted in December 2005 to allow time for breeding and shipping mouse lines to USAMRIID.					
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Introduction

Ricin, a biological toxin, belongs to the type II ribosome inactivating proteins which are heterodimeric glycoproteins that contain a toxophoric A-chain and a lectin B-chain joined together by a disulfide bond. Biological toxins act in concert with various (mostly unknown) host proteins (specific host proteins are essential for toxic action). The ricin toxin requires specific cellular proteins as it enters the cell, is processed, and moves to its target. This study investigates the effects of inactivating one or more of these cellular proteins in the context of the ricin toxin. Specifically, this study evaluated 278 mammalian genes for their involvement as host proteins contributing to the pathogenesis of ricin toxicosis using mouse gene knockout models. All host proteins analyzed in this study were “druggable,” meaning they belong to classes of proteins amenable to pharmaceutical intervention such as receptors, enzymes, channels, membrane and secreted proteins, transporters, etc. In an effort to identify the key host proteins required in ricin toxicosis, knockout mouse lines (278), each lacking a specific gene corresponding to the human homologous gene of interest, were constructed and evaluated for resistance or modification of the response to ricin exposure. Primary skin fibroblasts from each knockout line were challenged with ricin. In cooperation with scientists at the U.S. Army Medical Research Institute of Infectious Diseases, specific whole animal gene knockout models are also being made available for testing of modified responses to ricin toxin. This approach is designed to identify new drug targets for potential pharmaceutical intervention to treat ricin toxicosis.

Body

Native ricin was purchased from Vector Laboratories. Mouse primary skin fibroblasts were prepared as outlined in the proposal from wild-type animals and a kill curve using Alamar Blue (Biosource) was established for cells exposed to ricin for 24 hrs. This curve was found to be robust and reliable (see Figure 1).

Two hundred and seventy-eight mammalian genes were examined in the mouse model – corresponding fibroblast cell cultures were challenged in triplicate (3 separate homozygous knockout mice) with ricin at different points upon the kill curve. Similarly, wild-type cells were challenged simultaneously. Sample data is presented in Figure 1 in the appendix.

Specific tasks in the Statement of Work:

Task I. Gene selection

- A. 278 genes were selected for challenge based upon criteria developed by Lexicon Genetics to identify genes that might encode pharmaceutically tractable proteins.
- B. A full phenotypic analysis of all knockout mouse lines (278) was performed as described in the proposal, including studies of blood chemistry, hematology, metabolism, neurological responses, immunological functions, cell proliferation assays, and other medically relevant studies.
- C. Fibroblast cell cultures were established for all 278 knockout mice.

Task II. Ricin challenge

- A. In consultation with Dr. Luis DaSilva (Ft. Detrick), a kill curve was established for the mouse fibroblast cells.
- B. Two hundred and seventy-eight mammalian genes were examined in the mouse model. Thus, fibroblast cell cultures were challenged in triplicate (3 separate homozygous knockout mice) with ricin at different points upon the kill curve. Similarly, wild-type cells were challenged simultaneously. Sample data is presented in Figure 1.
- C. Some fibroblast cell cultures were retested; though none had strongly resistant phenotypes, at least one gene may have a potential role in ricin toxicity.

Task III. Construction of five knockout mouse lines for delivery to USAMRIID

Lexicon Genetics Incorporated has entered into a cooperative research and development agreement with USAMRIID (Dr. DaSilva's laboratory). The separate animal protocol was reviewed and approved by Lexicon's IACUC and is awaiting approval by the USAMRMC Animal Care and Use Review Office (ACURO).

In December 2005, Lexicon Genetics requested and was granted (from Contract Specialist, Mark Lohrmann) a 12-month extension of the performance period for this award. This extends the research period to December 14, 2006. The purpose for this extension is to allow time to breed and send five mouse lines to Dr. DaSilva, and to permit the appropriate time for Dr. DaSilva to complete the study.

Key Research Accomplishments

- An in vitro, colorimetric, ricin toxicity assay in mammalian genes was established and verified as robust.
- 278 genes were examined (mouse model) for contribution to ricin toxicosis.
- The 278 genes were determined to play little or only a modest role in ricin toxicosis (see Conclusions).

Reportable Outcomes

All ricin toxicity data was transferred to Dr. Luis DaSilva (USAMRIID). Additionally, Lexicon Genetics Incorporated has entered into a cooperative research and development agreement with USAMRIID (Dr. DaSilva's laboratory) to test five different knockout mouse lines against aerosolized ricin challenge, and for microarray experiments with exposed animals. Data will be reported in the final report under this award.

Conclusions

The 278 genes that were evaluated play little or only a modest role in ricin toxicity, as judged by the in vitro test of fibroblast cultures. Genes exhibiting at least a modest resistance or modification in response to ricin exposure were retested. Among those genes retested, at least one gene may have a potential role in ricin toxicity as the cytotoxicity assay demonstrated a modest increase in survivability. In vivo studies to further investigate this potential (including other gene candidates) are being planned in cooperation with Dr. DaSilva. A manuscript discussing the preliminary data is currently in preparation. Additional genes should be assayed to ascertain suitable candidates for pharmaceutical intervention.

Appendices

Figure 1.

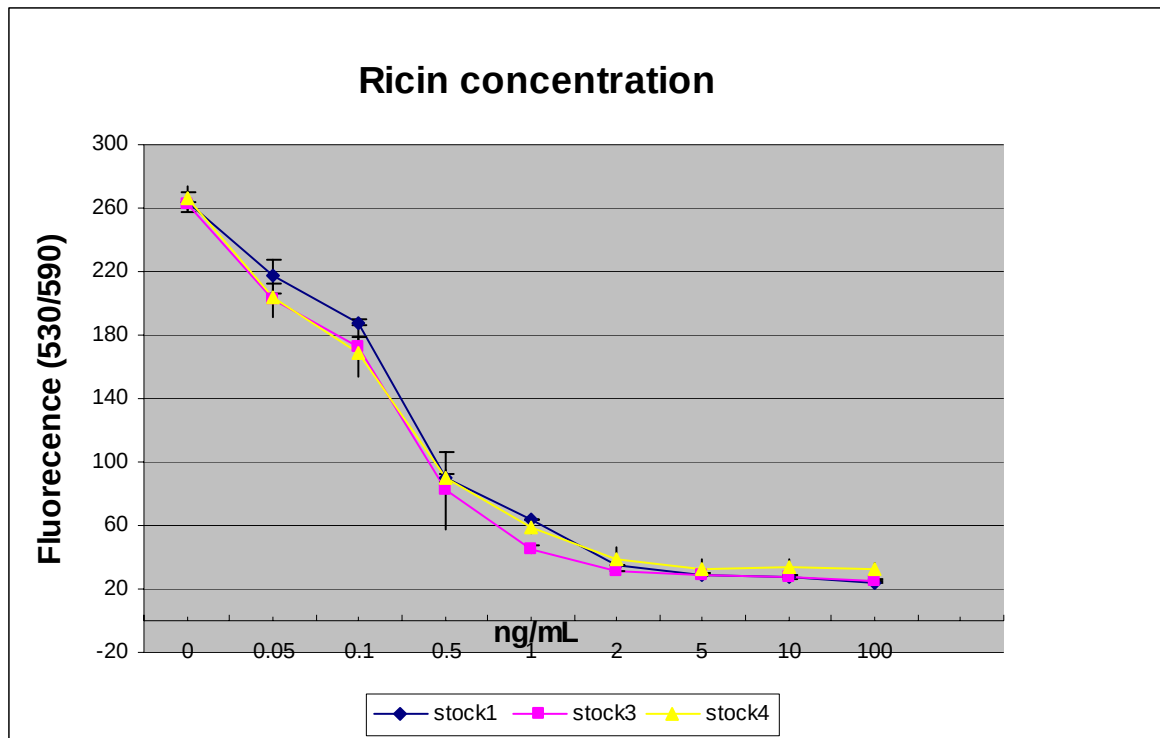


Figure 1. Primary skin fibroblasts were exposed to varying concentrations of ricin as described in the proposal. Reduced fluorescence is a measure of cell death. Cells exposed to buffer only or staurosporine were used as controls (data not shown here).