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Anti-Ricin Efficacy Comparison of a Humanized Therapeutic Antibody Produced in Plants with Its Mammalian Cell-Produced Counterpart

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Abstract

Ricin is regarded as a terrorist risk for the public due to its high toxicity and ease of production. Currently, there is no therapeutic antibody or vaccine available against ricin. Our previously reported humanized recombinant antibody, hD9, demonstrated promising post-exposure therapeutic activity against ricin intoxication. To reduce the manufacturing cost, the production of hD9 has been explored in plants. hD9 was transiently expressed, along with human galactosyl transferase, in a line of Nicotiana benthamiana (ΔFX) with suppressed expression of the plant-specific glycosyltransferases (xylosyl- fucosyl-transferases) to mimick human glycosylation. The plant-produced hD9 (PhD9) was successfully purified and polished; formulated product was 97% pure and contained ≤1 endotoxin unit per mg of PhD9. Anti-ricin efficacy of PhD9 was compared with its mammalian cell-produced counterpart, hD9. In an in vitro Vero cell-based ricin neutralization assay, PhD9 demonstrated ricin neutralization potency comparable to hD9. In a ricin intoxication mouse model, an intraperitoneal injection (5 µg) per mouse of either PhD9 or hD9 rescued 100 and 50% of the mice 4 and 6 hrs after i.p. challenges with 5×LD_{50} doses, respectively. Thus, PhD9 is comparable to hD9 in terms of efficacy against ricin intoxication both in vitro and in vivo.

Introduction

Ricin is extracted from the common caster bean and has the potential to be used as an biothreat agent. Ricin can enzymatically cleave ribosomal RNA to stop protein synthesis (Fig 1). Antibodies against ricin can neutralize the toxin (Fig.2). In our previous studies, a highly efficacious anti-ricin monoclonal antibody was developed and successfully humanized.

Therapeutic antibodies are among the most expensive drugs, leading to extremely high production costs ($300/gram). The high cost has dramatically affected the development of antibodies as therapeutics. Plant systems are fast, efficient, highly versatile (for new product development), and easily scalable with significantly reduced manufacturing costs. In addition, they are free from contamination by mammalian pathogens. In this study, the humanized anti-ricin, hD9 was produced in plants and then the plant-produced hD9 (PhD9) was evaluated both in vitro and in vivo in terms of anti-ricin efficacy as compared with its mammalian cell-produced counterpart, hD9.

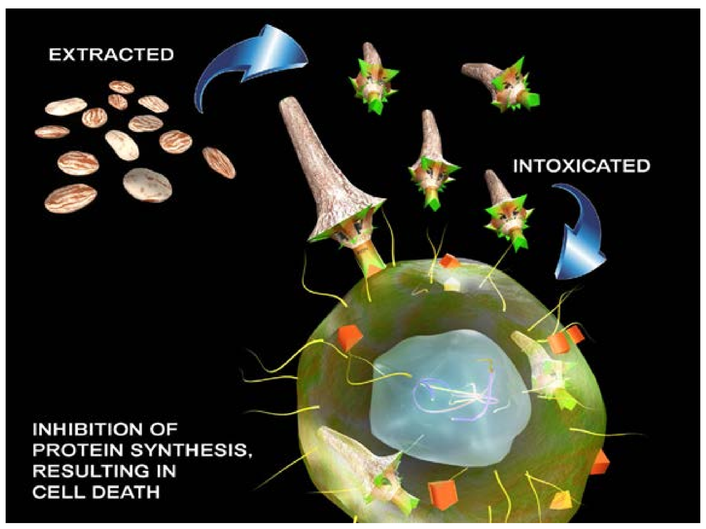

Fig.1 Mechanism of ricin toxicity. Ricin toxin derived from the beans of the castor plant is listed as a Category B Agent by the Centers for Disease Control and Prevention.

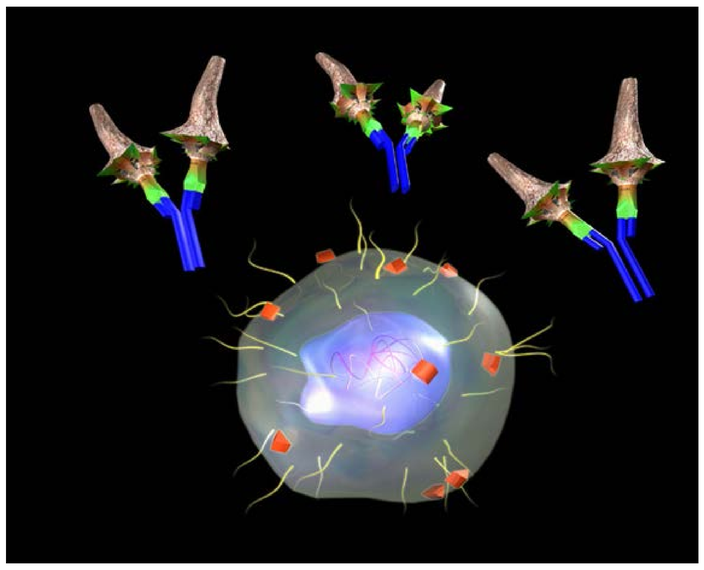

Fig. 2 Mechanism of anti-ricin protective antibodies. Protective anti-ricin antibodies can block ricin binding to cells.

Results

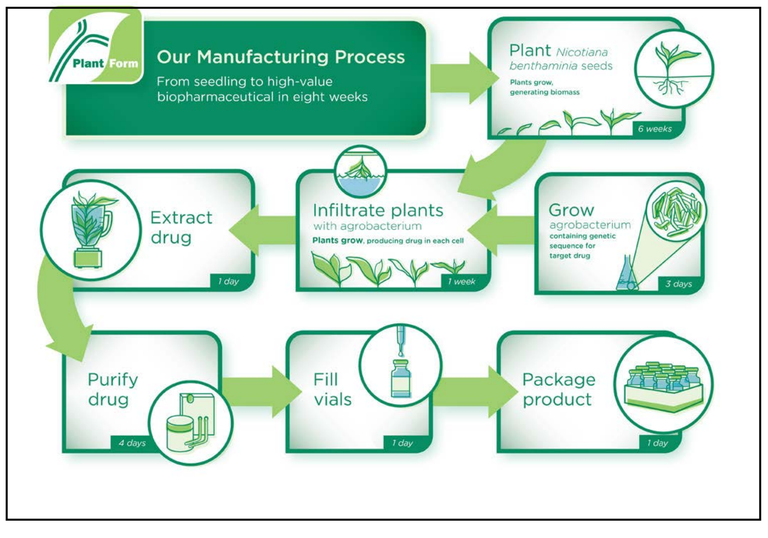

Fig. 3 Production of PhD9 in plants. PhD9 was expressed in Nicotiana benthamiana (closely related to smoking tobacco) using a rapid transient expression system that results in expression levels greater than 200 mg /kg of fresh weight with a humanized glycosylation pattern. After purification, a total of 350 mg of PhD9 with endotoxin levels as low as 1 EU/mg was delivered.

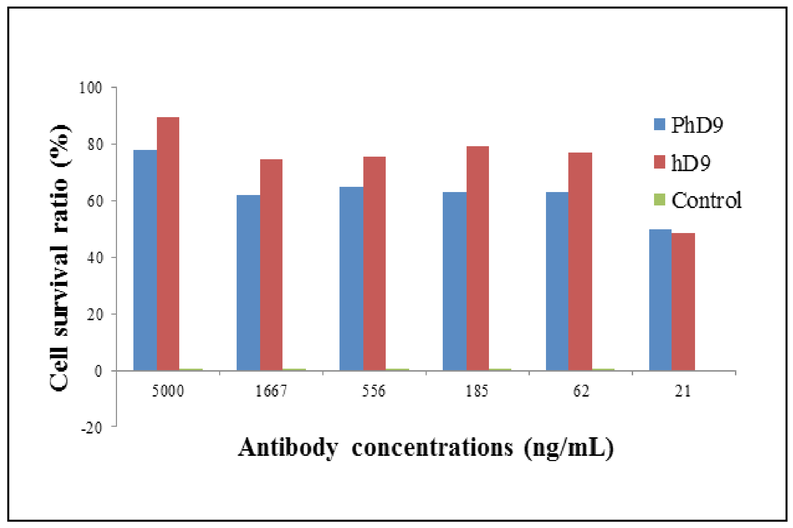

Fig. 4 In vitro neutralization assay. A Vero cell toxicity neutralization assay with Alamar blue as an indicator was performed in 96-well plates. Ricin was incubated with a serial dilution of PhD9, hD9, or an unrelated antibody (control) for 2 hrs at 37 °C. Vero cells were added into the ricin antibody mixture. After incubation at 37 °C, 5% CO_2 for 2 days, Alamar blue was added and incubated for 6-7 hours. On a plate reader, the plate was read at an absorbance of 570 nm with 600 nm as a reference.

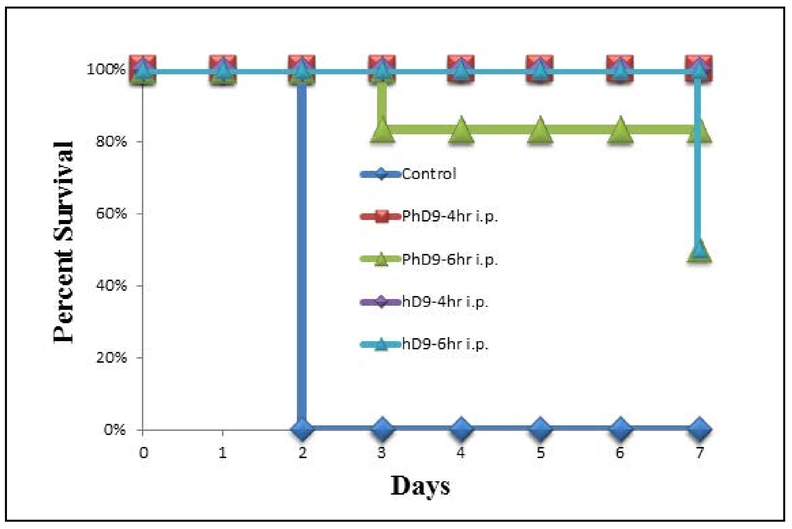

Fig. 5 In vivo protection assay. Groups of 5-8 Balb/c female mice (4-6 weeks old) were intraperitoneally challenged with 5×LD_{50} ricin and 5 µg per mouse of PhD9, hD9, or an unrelated antibody (control) was intraperitoneally administered to mice at 4 or 6 hr post-ricin challenge. The mice were observed for morbidity and mortality over two weeks.

Conclusion

The PhD9 is comparable to its mammalian cell-produced counterpart, hD9 in terms of efficacy against ricin intoxication, indicating that plants can be used as bioreactors for a fast, efficient, and cost-effective platform for large-scale production of hD9.

Furthermore, development of this production platform in plants would provide DRDC the capability for cost-effective and large-scale production of any protein-based medical countermeasures against biothreat.

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