CLINICAL IMPLICATIONS OF BASIC RESEARCH

# Novel Proteins to Neutralize Venom Toxins

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Snakebite envenomation is a neglected tropical disease that kills and maims hundreds of thousands of people every year, particularly in sub-Saharan Africa, Asia, and Latin America.[1] The mainstay of treatment of envenomation is the parenteral administration of antivenoms made of immunoglobulins or their fragments that have been purified from the blood plasma of animals (typically horses or sheep) that have been immunized with snake venoms. When manufactured under Good Manufacturing Practices, produced with appropriate venom mixtures, and administered expeditiously, antivenoms have good safety and efficacy profiles, especially with regard to abrogation of the systemic life-threatening effects of envenomation. However, antivenoms induce adverse effects in some patients, have low efficacy against toxins with poor immunogenicity (see Key Concepts) (e.g., neurotoxins and cytotoxins [toxins that induce tissue damage]), and should be administered only in health care facilities.[2] To circumvent these limitations, there is a growing interest in novel approaches to the treatment of snakebite envenomation, including the use of human recombinant antibodies,[3] repurposed synthetic inhibitors of toxins,[2], or combinations of antibodies and inhibitors.[3]

Of interest, then, is a study reported by Vázquez Torres et al.[4] They used a powerful computational deep-learning method (RoseTTAFold diffusion [RFdiffusion]) that enables the design of novel proteins (i.e., computational design of a protein with a sequence and structure that do not correspond to those of any known protein) with high precision.[5] To show the potential of this tool to generate effective inhibitors of snake-venom toxins, the researchers selected as targets some potent toxins that belong to the so-called "three-finger" toxin family.[4] These toxins are present in the venoms of snakes of the Elapidae family (e.g., cobras, kraits, mambas, and sea snakes, among others). Some of these toxins are powerful neurotoxins that bind to and block the nicotinic cholinergic receptors in muscle fibers, causing neuromuscular blockade and paralysis. Other snake-venom toxins inflict severe tissue necrosis, often leading to permanent sequelae (Fig. 1A).[1] Three-finger toxins have a low molecular mass and are therefore poorly immunogenic, thus presenting a chal-

## KEY CONCEPTS

**Immunogenicity**
The ability of a substance such as a protein to trigger an immune response.

**Nicotinic cholinergic receptors**
A family of receptors located in the central nervous system, peripheral ganglia of the autonomic system, and skeletal muscle. In skeletal muscle cells, these receptors are concentrated in the motor end-plate region of the plasma membrane (the part adjacent to presynaptic membrane of the efferent [motor] neuron). These receptors bind the neurotransmitter acetylcholine released from the nerve terminals of efferent neurons during neuromuscular transmission. The receptors are ligand-gated ion channels which, on the binding of acetylcholine, undergo conformational changes that result in an increase in the permeability to cations. Membrane depolarization ensues, which may lead to an action potential and voluntary skeletal muscle contraction.

**X-ray crystallography**
An experimental platform that allows the study of the three-dimensional structure of small and large molecules, including macromolecules such as proteins. Purified proteins are crystallized, and an x-ray beam is applied to the crystals. The rays are diffracted by the electron clouds of the atoms present in the crystal. The crystals are gradually rotated to generate additional reflection patterns. A large set of data is collected during the process, followed by computational analysis, and angles and intensities of the diffracted x-rays are calculated. Additional refinement analyses are made to generate a three-dimensional structure of the molecule.

*An illustrated glossary is available at NEJM.org*



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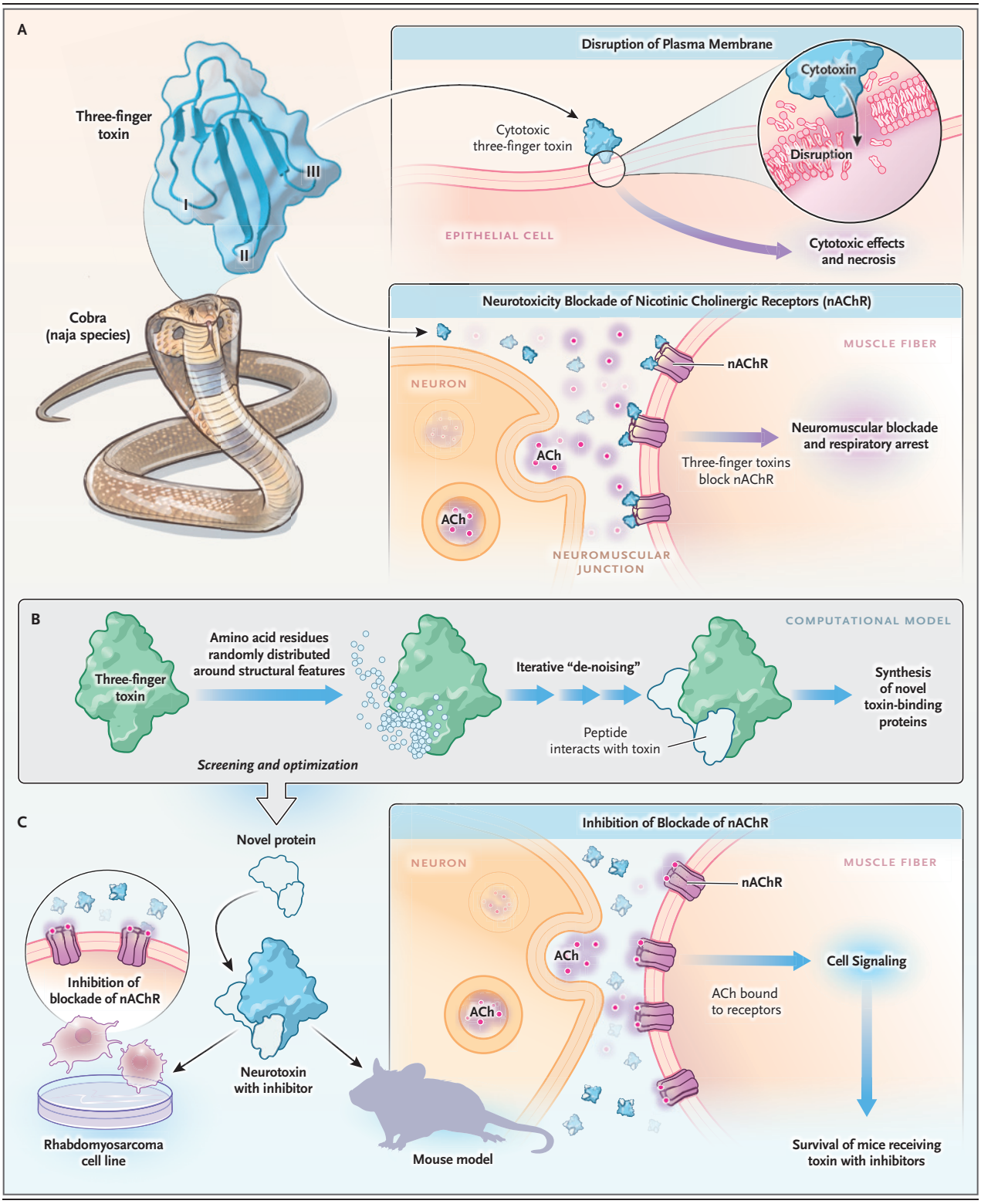




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**Figure 1 (facing page). Inhibition of Cobra-Venom Toxins with Novel Proteins.**

Cobra venoms are enriched with toxins of low molecular mass, known as three-finger toxins (Panel A). Some of these are neurotoxins, which bind with high affinity to the nicotinic cholinergic receptors in muscle fibers, causing neuromuscular blockade and respiratory arrest. Other three-finger toxins are cytotoxins, which disrupt the integrity of the plasma membrane of cells, causing necrosis. Vázquez Torres et al.[4] recently described the design of novel protein inhibitors, beginning with a computational model in which amino acid residues were randomly distributed around the structural determinants of toxins (Panel B). This step was followed by iterative "de-noising" steps (part of the computational deep-learning method), which led to folded structures that were predicted to have high affinity to each target toxin. Synthetic genes encoding these inhibitor proteins were then prepared, and the inhibitors were expressed and tested for their binding affinity to toxins and their ability to abrogate toxic effects in cell lines and in mice (Panel C). Complete neutralization of neurotoxins was observed in patch-clamp experiments in a rhabdomyosarcoma cell line expressing the nicotinic acetylcholine receptor. Inhibition of two neurotoxins was assessed in a lethality assay in a mouse model. Mice that received the toxins through intraperitoneal injection died, whereas those that received the toxins that had been preincubated with the relevant inhibitor survived. When the inhibitors were administered 15 minutes after injection of the toxins, tered 30 minutes after injection of the toxins, most survived. None of the surviving mice had evidence of limb or respiratory paralysis. ACh denotes acetylcholine.

lenge to the generation of high antibody titers in animals that are immunized with venoms that contain these toxins.

To generate inhibitors of these toxins, Vázquez Torres et al. used computational modeling to design a series of proteins (Fig. 1B). On the basis of the amino acid sequences of the proteins, synthetic genes were then designed and prepared for the most promising candidates, and those inhibitors with the greatest binding affinities for the toxins were optimized by means of iterative screening. The inhibitor proteins were then expressed in a bacterial system and tested.[4] Analysis by x-ray crystallography showed that the structures of these best-candidate inhibitors, when complexed with the toxins, were similar to those of the models designed with the use of computational modeling. When these inhibitors were tested for abrogation of toxicity with the use of in vitro models (i.e., blockade of neurotoxins in a rhabdomyosarcoma cell line and blockade of venoms and a cytotoxin in a keratinocyte cell line), the inhibitors proved effective. When tested in mouse models, the inhibitors protected mice from the paralyzing and lethal effects of the neurotoxins when the inhibitors were incubated with the neurotoxins before injection and when the inhibitors were administered 15 minutes or 30 minutes after toxin injection (a scenario that models the natural circumstances of envenomation) (Fig. 1C).

These findings open up possibilities for the development of venom inhibitors based on machine-learning tools. Such inhibitors can be designed to be thermostable and can be synthesized in large quantities. The versatility of this method allows the design of proteins that act as inhibitors against different types of toxins with key roles in envenomation (including phospholipases $A_2$, metalloproteinases, and serine proteinases in addition to three-finger toxins). Our growing understanding of the composition and toxicity of snake venoms is enabling the identification of candidate toxins in different venoms for the design of inhibitors. However, the complexity of snake venoms poses a challenge, because toxins — even those of the same family — differ in structure, and a variety of protein inhibitors may be needed for effective neutralization, particularly of venoms made up of a cocktail of toxins.

From a pharmacokinetic perspective, novel venom inhibitors should have large volumes of distribution to reach different tissue compartments and block toxins acting on cells, neuromuscular junctions, or extracellular matrixes. They should have a prolonged half-life in the bloodstream so that toxins that reach the circulatory system later in the course of envenomation are neutralized. This variety of demand suggests that inhibitors of different molecular formats and sizes, with different pharmacokinetic profiles, may be needed. An interesting possibility is the enrichment of animal-derived antivenoms with complementary novel inhibitors that block toxins that are poorly neutralized by existing antivenom treatments.[2,4]

Challenges to the clinical deployment of these new types of venom inhibitors include in-depth preclinical evaluation based on in vitro and in vivo models; the design, organization, and imple-



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mentation of clinical trials to assess safety and efficacy; the development of regulatory frameworks for approval of the new inhibitors; the commitment of manufacturers to large-scale production; and the implementation of public health policies to guarantee availability and accessibility, especially in regions where the incidence of envenomation is high.[2] It is likely that the future landscape of the treatment of snakebite envenomation will involve the coexistence of animal-derived antivenoms, recombinant antibodies, and inhibitors of various types, including novel proteins, with variations depending on the target venom to be neutralized and on regional contexts.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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**1.** Gutiérrez JM, Calvete JJ, Habib AG, Harrison RA, Williams DJ, Warrell DA. Snakebite envenoming. Nat Rev Dis Primers 2017;3:17079.
**2.** Gutiérrez JM, R Casewell N, Laustsen AH. Progress and challenges in the field of snakebite envenoming therapeutics. Annu Rev Pharmacol Toxicol 2025;65:465-85.
**3.** Glanville J, Bellin M, Pletnev S, et al. Snake venom protection by a cocktail of varespladib and broadly neutralizing human antibodies. Cell 2025 April 30 (Epub ahead of print).
**4.** Vázquez Torres S, Benard Valle M, Mackessy SP, et al. De novo designed proteins neutralize lethal snake venom toxins. Nature 2025;639:225-31.
**5.** Watson JL, Juergens D, Bennett NR, et al. De novo design of protein structure and function with RFdiffusion. Nature 2023;620:1089-100.

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