

Obesity, Fitness & Wellness

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Researchers describe findings in anthrax studies

Anthrax research advances have been reported from the United States.

Study 1: According to recent research from the United States published in the journal *Proceedings of the National Academy of Sciences of the United States of America*, translocation of **anthrax** toxin's lethal factor (LF) is initiated by entry of its N-terminus into the protective antigen channel.

"Entry of the enzymatic components of **anthrax** toxin LF and edema factor into the cytosol of mammalian cells depends on the ability of the activated protective antigen (PA63) component to form a channel (pore) in the membrane of an acidic intracellular compartment," wrote **S. Zhang** and colleagues, Harvard University. "To investigate the mechanism of translocation, we characterized N-terminally truncated forms of the PA63-binding domain of LF (LFN)."

The data showed that "deleting 27 or 36 residues strongly inhibited acid-triggered translocation of LFN across the plasma membrane of CHO-K1 cells and ablated the protein's ability to block PA63 channels in planar lipid bilayers at a small positive voltage (+20 mV)."

"Fusing a H6-tag to the N terminus of the truncated proteins restored both translocation and channel-blocking

activities," continued the **researchers**. "At +20 mV, N-terminal H6 and biotin tags were accessible to Ni and streptavidin, respectively, added to the trans compartment of a planar bilayer.

"On the basis of these **findings**, we propose that the N-terminus of PA63-bound LF or edema factor enters the PA63-channel under the influence of acidic pH and a positive transmembrane potential and initiates translocation in an N- to C-terminal direction," concluded the authors.

Zhang and colleagues published their **study** in *Proceedings of the National Academy of Sciences of the United States of America* (Evidence that translocation of **anthrax** toxin's lethal factor is initiated by entry of its N terminus into the protective antigen channel. Proc Natl Acad Sci USA, 2004;101(48):16756-16761).

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Study 2: Researchers have conducted a Western blot analysis of the exotoxin components from *Bacillus anthracis* separated by isoelectric focusing gel electrophoresis.

"The components of the *Bacillus anthracis* exotoxins, protective antigen (PA), lethal factor (LF), and edema factor (EF), from 24 isolates were separated by isoelectric focusing gel electrophoresis and detected by Western blot with monoclonal antibodies. Only two isoforms each were observed for PA and EF. Four isoforms were identified for LF," scientists **in** the United States report.

"The biological activities of both lethal toxin and edema toxin were measured by using **in vitro** cell-based assays. This **study** provides another method of characterizing various isolates of *B. anthracis* by determining the isoelectric points of the exotoxin components and may be useful **in** the development of protective vaccines against *B. anthracis* infection," wrote Stephen F. Little at the United States Army Medical Research Institute of Infectious Diseases.

Little published his **study in** *Biochemical and Biophysical Research Communications* (Western blot analysis of the exotoxin components from *Bacillus anthracis* separated by isoelectric focusing gel electrophoresis. *Biochem Biophys Res Commun*, 2004;317(1):294-300).

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Study 3: Anthrax lethal factor-cleavage products of mitogen-activated protein kinase (MAPK) kinases exhibit reduced binding to their cognate MAPKs.

According to published research from the United States, "**Anthrax** lethal toxin is the major cause of death **in** systemic **anthrax**. Lethal toxin consists of two proteins: protective antigen and LF (lethal factor). Protective antigen binds to a cell-surface receptor and transports LF into the cytosol.

"LF is a metalloprotease that targets MKKs [MAPK (mitogen-activated protein kinase) kinases]/MEKs [MAPK/ERK (extracellular-signal-regulated kinase) kinases], cleaving them to remove a small N-terminal stretch but leaving the bulk of the protein, including the protein kinase domain, intact," stated A. Jane Bardwell and colleagues at the University of California-Irvine. "LF-mediated cleavage of MEK1 and MKK6 has been shown to inhibit signaling through their cognate MAPK pathways. However, the precise mechanism by which this proteolytic cleavage inhibits signal transmission has been unclear.

"Here we show that the C-terminal LF-cleavage products of MEK1, MEK2, MKK3, MKK4, MKK6, and MKK7 are impaired **in** their ability to bind to their MAPK substrates, suggesting a common mechanism for the LF-induced inhibition of signaling," concluded Bardwell and her collaborators.

Bardwell and her coauthors published their **findings in** the *Biochemical Journal* (**Anthrax** lethal factor-cleavage products of MAPK (Mitogen-activated protein kinase) kinases exhibit reduced binding to their cognate MAPKs. *Biochem J*, 2004;378(Part 2):569-577).

Additional information can be obtained

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The information **in** this article comes under the major subject areas of **Anthrax**, Biowarfare, Bacteriology, and Proteomics.

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