Healthcare industry BW

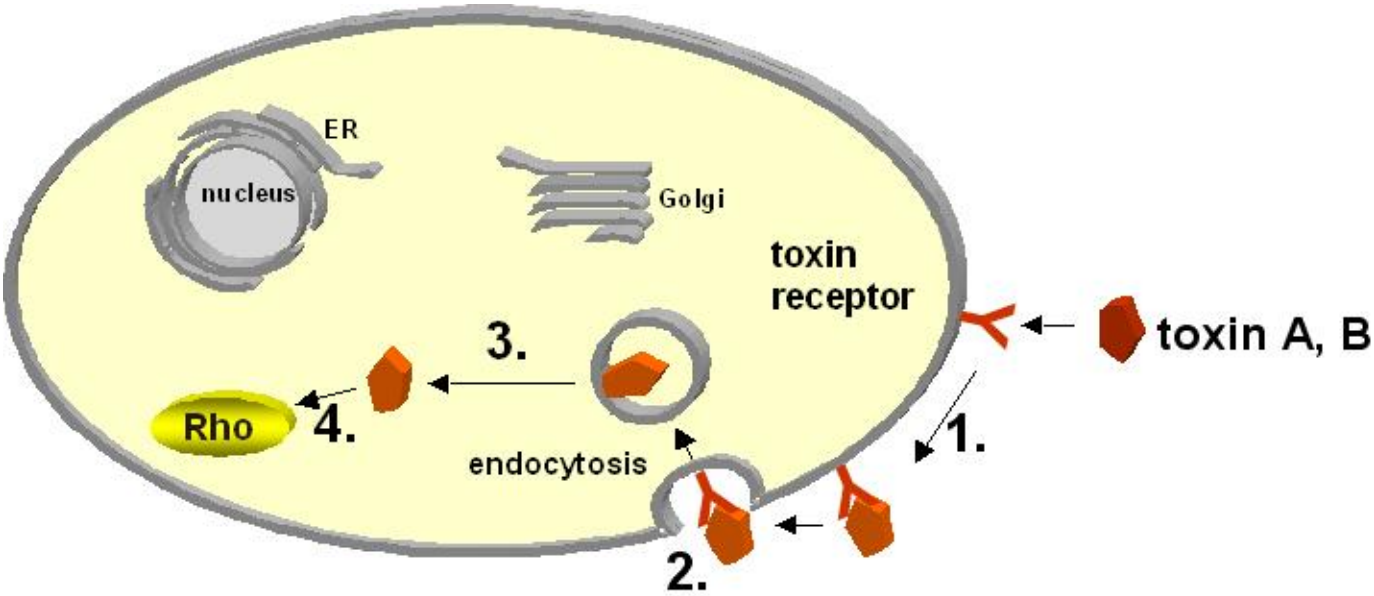
Helpful toxins

Sometimes, a few nanogrammes of toxin are all that is needed to kill someone. Prof. Dr. Dr. Klaus Aktories from the Institute of Experimental and Clinical Pharmacy at the University of Freiburg is investigating why some bacterial toxins are so extremely toxic. Together with his team of researchers, Aktories has identified the molecular mechanism of action of Clostridium difficile toxins. Such toxicological knowledge is not only helpful in clinical applications, but is also important in the laboratory.

Clostridium difficile infections often occur in hospitalised patients who have been treated with antibiotics. Antibiotics destroy the normal bacterial flora of the bowel allowing the growth of Clostridia, which produce toxins and cause diarrhoea (antibiotic-associated diarrhoea). In some patients, this leads to inflammation of the intestines (pseudomembranous colitis). In elderly patients, C. difficile colitis infections can be severe, or even fatal. "In recent times, particularly aggressive Clostridium-difficile bacteria have been observed that have led to a severe progression of disease and increased mortality," said Prof. Aktories. It is assumed that this is caused by a new, hypervirulent C. difficile strain that is resistant against the antibiotics of the fluorochinolon group. This strain also produces larger amounts of toxin.

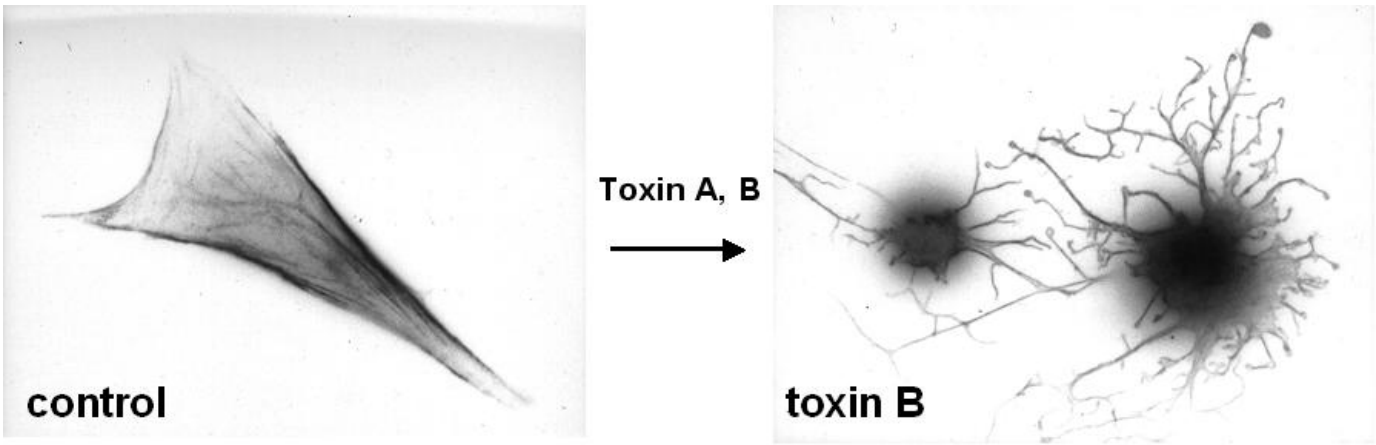
A complicated mechanism of action

Diseases caused by Clostridium difficile are mainly the result of two toxins (toxin A and B) produced by the bacteria. Aktories and his colleagues have spent the last few years finding out how the toxins enter the cells of the bowel mucosa and other cells and which cellular processes are affected. They have recently found out that the toxins are proteins with four subunits. One subunit is required by the toxin to attach to the membrane of the target cells. If the toxin succeeds in attaching itself, then the target cell forms small bubbles (endosomes) around the toxin and incorporates it. The acidification of the endosomes, a process that also happens under normal physiological conditions, alters the structure of the toxin, a second subunit turns to the outside and integrates into the membrane of the endosome to form a pore. The mechanism of pore formation is still unknown. The third subunit is a protease that cleaves the toxin to release a fourth subunit into the cell's interior – mostly likely through the pore. "The fourth subunit is the biologically active part of the toxin," explains Aktories.



The schematic shows how the Clostridium difficile toxins A and B enter their target cell. (Figure: Prof. Dr. Dr. Klaus Aktories)

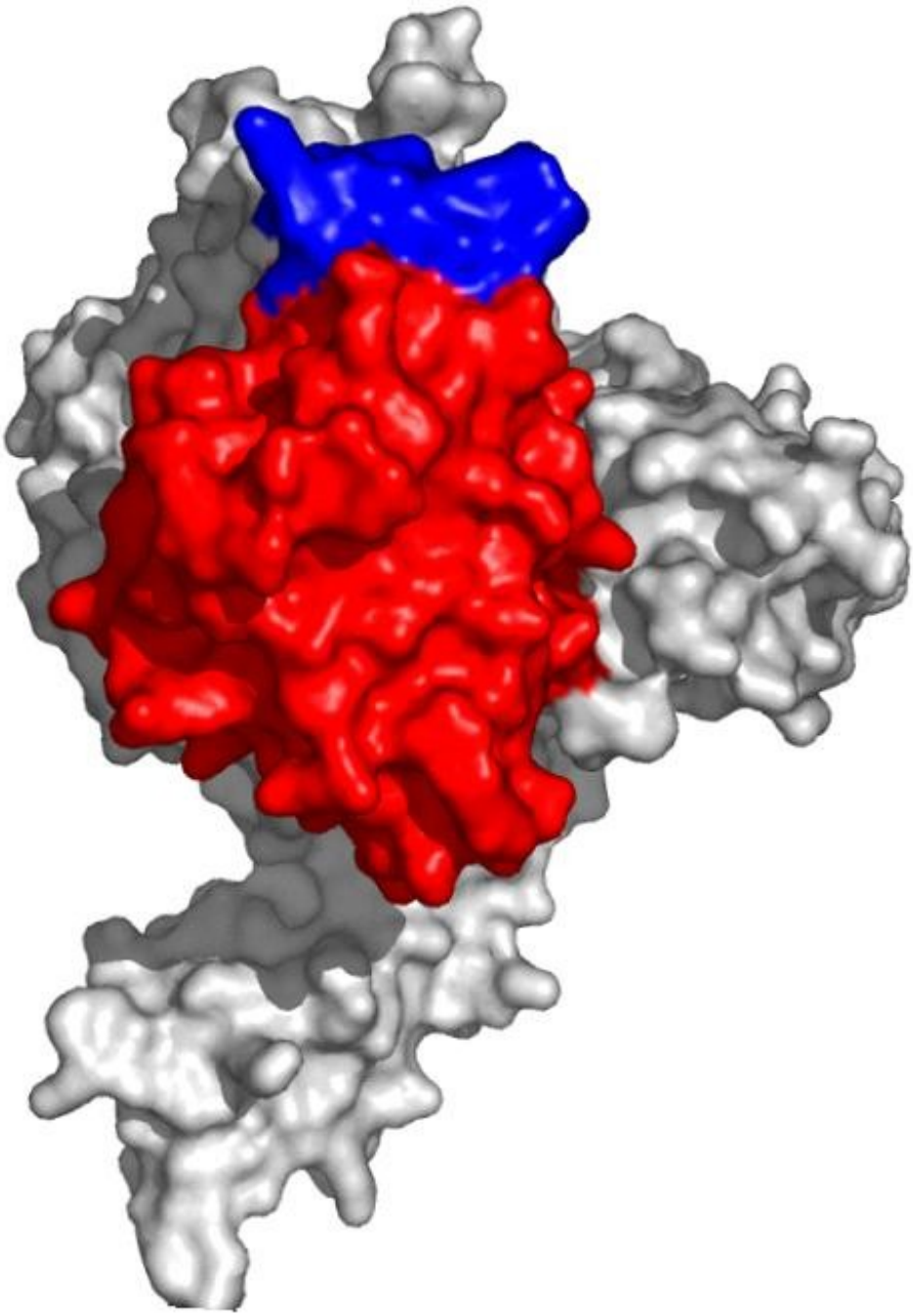
Working together with the Institute of Organic Chemistry and Biochemistry, Aktories's group has crystallised this part of the toxin and elucidated its structure. Using molecular biology methods, the researchers identified the targets of the toxin's active subunit inside the cells. They have found out that this subunit attaches a sugar molecule to Rho, which is an important molecular switch. The attachment of a sugar molecule inactivates Rho and prevents important signalling pathways in the cell's interior from being switched on or off. This disturbs the balance of many cellular processes. For example, the cytoskeleton (skeleton of the cell) does not form in the normal way, many genes are no longer transcribed correctly, the signalling networks in the immune cells break down and reduce the cell's ability to fend off pathogens. Also the cells of the intestinal mucosa lose contact with their neighbours, which makes the intestinal wall permeable and causes the typical symptoms of diarrhoea. "Now that we know the structure of this toxic, active subunit, we are able to look specifically for effective inhibitors," said Aktories adding that "we will also be able to put these findings to good use in the laboratory."



The Clostridium difficile toxins A and B affect, amongst other things, the cytoskeleton. As a result, the cell loses its normal shape. (Photo: Prof. Dr. Dr. Klaus Aktories)

Switching off Rho

The example of Clostridium botulinum neurotoxins shows that the knowledge about the mechanism of action of bacterial toxins is not only of interest for clinical applications. These toxins specifically attack the synapses between the nerve cells and the muscles and prevent the release of the neurotransmitter acetylcholin. This leads to muscle weakness. The respiratory muscles are also affected, which may cause death due to respiratory problems. These are signs of botulism. According to Aktories, botulinus neurotoxins are among the most toxic in the world. A few nanogrammes are sufficient to kill people; 10 picogrammes of botulinum toxin can kill a mouse.



The Clostridium difficile (grey) toxin B binds to the molecular switch Rho (red and blue). (Figure: Prof. Dr. Dr. Klaus Aktories)

Nevertheless, neurotoxins are also used as drugs, in particular against muscular cramps and spasms, under the condition that only small doses are used (rule: "the dose makes the toxin"). In addition, the neuroscientists have used the dangerous substances to identify the mechanism that

leads to the release of neurotransmitters at synapses. Neurotoxins specifically degrade the proteins involved in this process. The lack of these proteins provides the researchers with important insights into their normal function.

"The Clostridium difficile toxins A and B can be used for similar experiments," said Aktories. "In future, we will be able to switch the molecular switch Rho off and gain detailed insights into its function."

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Further informaiton:
Prof. Dr. Dr. Klaus Aktories
University of Freiburg
Institute of Experimental and Clinical Pharmacology and Toxicology
Albertstr. 25
79104 Freiburg
Tel.: +49-(0)761/2035301
Fax: +49-(0)761/2035311
E-mail: klaus.aktories@pharmakol.uni-freiburg.de

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