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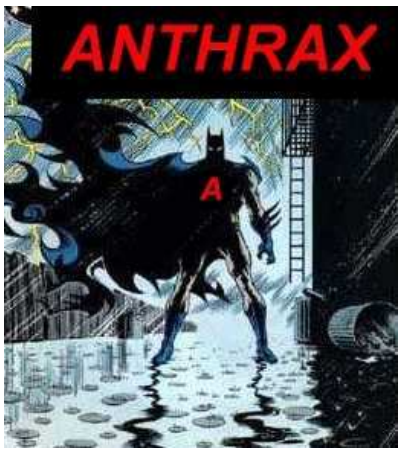
Anthrax Toxins: A Diabolical Threesome



Anthrax has been involved in a recent wave of crimes in the United States. Long known as a livestock disease, it has come to the forefront today as a biological weapon. Behind the name - which sounds as though it has been picked out of a 1950 comic strip - there is a bacterium and behind the bacterium, there lies a fearsome threesome of proteins.

Until recently, not many people knew what anthrax was. However, the disease has long been an object of intense interest to specialized military research teams which develop biological weapons. All because of particular properties associated with the bacterium and with the dangerous toxins it produces.

Bacillis Anthracis: common, but very tough

Anthrax[1] is a bacterial disease caused by *Bacillus Anthracis*, which was discovered in 1863 by the French zoologist Casimir-Joseph Davaine. In a hostile environment like soil, the anthrax bacterium can transform into a spore. Thus protected by a rigid shell, it becomes extremely resistant to variations in temperature, acidity as well as disinfectants, and can survive in this form for several years. But in a rich environment – such as the blood of an animal or a human being – it multiplies rapidly.

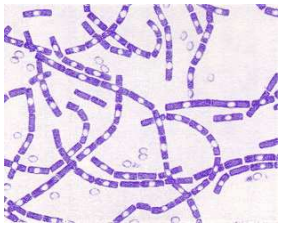


Fig.1 *Bacillus Anthracis*

Anthrax's usual victims are domestic herbivores like cattle, oxen, sheep or goats. The bacteria live in the soil and animals are infected while ingesting grass, fodder or water contaminated by anthrax

[1] From the Greek 'charcoal' and so named because of the blackening of the blood, and the appearance of deep black sores on the skin in cutaneous anthrax.

spores. If an animal has a lesion in its mouth, the spores can invade the bloodstream and "germinate", thereby shedding their shells and multiplying. The infection quickly turns into fatal blood poisoning marked by fever, an edema of the pharynx and a blackening of the blood – hence the name of the illness relating to the color of charcoal[1].

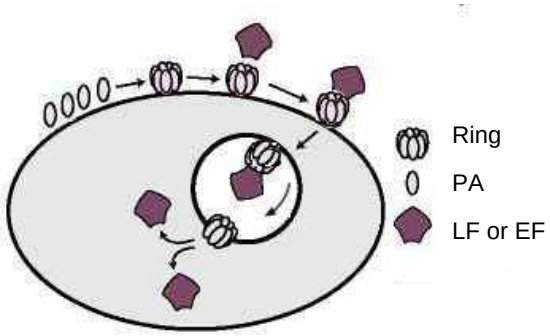
People can also contract anthrax via contact with sick animals but also and by manipulating corpses. Hence, vets, breeders and tanners are on the front line. *Bacillus Anthracis* can enter the body through any one of three different "doors", each of which produces a different form of the disease. Spores can penetrate the skin via any type of skin lesion; this is the most common form of anthrax (95% of all cases), and is relatively easy to treat and only exceptionally fatal. It is the pulmonary form — caught by inhaling the bacteria — which is the most serious. Finally, there is also a digestive form which can be caught by eating contaminated meat – but this is extremely rare.

Three proteins for an exemplary mechanism

Bacillus anthracis gets most of its virulence from two cell-killing toxins it produces. These toxins are themselves made up of three proteins with particularly evocative names; the Protective Antigen (here abbreviated "PA"), the Lethal Factor (LF), and the Edema Factor (EF). Taken separately, not one of these proteins is toxic. However when the PA protein is associated with EF – the "edema toxin" – they cause the formation of edema, precisely. Similarly, when PA is associated with LF, together they form the "lethal toxin", which is responsible for the death of anthrax-infected people and animals.

Hence, the PA protein plays a central role in the disease. In fact, it uses quite an elegant mechanism to help the EF and LF proteins penetrate an organism's cells. In the first phase, a PA protein secures itself to the surface of a cell, by binding to a specific protein found on the cell membrane. Once "docked", PA can bind to other PA proteins also present on the surface of the cell membrane. In this way, seven PA proteins join together to form a ring-like structure. Such a ring can bind to either an EF protein or an LF protein, but not both at the same time. Once bound, the cell membrane forms a depression and the ring is engulfed into the cell's interior. As a result, the ring bound to either FL or FE ends up in a sort of sack on the inside of the cell. The ring then fuses into the sack's flank and forms a hole from which FL and FE are freed into the cytoplasm.

A protein which plays the role of a receptor for the PA protein has recently been identified. This will allow a more detailed investigation of the mechanism, and possibly lead to new therapeutic approaches. It is already known that once within the cell, LF and EF act differently; but the exact chain of events which finally causes the illness is still not clear.



Courtesy of Harvard Medical School

Fig.2 *Model of how the anthrax toxins enter a cell*

The LF protein is a protease. Similar to a selective pair of "molecular scissors", the role of a protease is to cut its target proteins at precise points. LF acts on a very important group of proteins, which form the basis for a complex communication system within the cell's interior: the MAPKKs[2].

To understand the purpose of such a communication system, imagine a cell as a sort of factory specialized in the production of proteins. Like any company, the cell has different departments which must communicate with each other to let the factory work efficiently. What is more, this particular "factory" also communicates with its environment, which sends it all kinds of information and commands in the form of chemical signals. Upon arrival within the cell, these messages must be sent on to the appropriate department. This is the job of the MAPKKs.

So in response to external signals, the MAPKKs trigger a cascade of chain reactions in the cell, which do not cease until the message is delivered to its destination. What LF does is inactivate certain MAPKKs by severing them at specific locations and thus blocking signal transmission. But it is likely that LF also acts on proteins not yet identified. EF also works on the internal signaling system by producing a small molecule within the cell — cyclic AMP — which is itself a kind of "internal messenger".

[2] Abbreviation of MAP kinase-kinase

Though the exact sequence of events which leads to the disease remains murky, it is obvious that the destruction or even just the disturbance of the cell's internal communication system can gravely affect its normal functioning.

A vaccine, yes — but not for everyone

Capable of decimating an entire flock in just a few days, anthrax was the stock breeders' curse. Between 1877 and 1881, Louis Pasteur and his colleagues studied the disease's epidemiology and demonstrated *Bacillus anthracis*' key role as a pathogen. The results of these studies led them to develop a type of vaccination. The so-named anti-charcoal vaccination is a "live" vaccination, meaning it contains a strain of the bacterium whose virulence has been attenuated. Thanks to the existence of such a vaccine, anthrax is a very rare illness today - which is exactly what made the recent human cases in the United States immediately suspicious.

In humans, the symptoms of the disease vary according to how it was contracted. When the infection is cutaneous, a kind of pustule forms around the lesion, which slowly turns into a blackish ulcer. Without treatment, the bacteria cause a subsequent infection of the blood which leads to septicemia. If the spores are ingested the disease is brutal and pernicious, with an onset of nausea, diarrhea and blood, rapidly followed by septic shock. If the spores are inhaled, the infection shows the same symptoms as those for a common cold accompanied by a fever, aches and a cough. The symptoms become rapidly worse causing severe respiratory distress and shock. Death follows about three days after the appearance of the first symptoms.

The only current treatment for anthrax is the prompt use of antibiotics. If these are administered quickly, *Bacillus anthracis* is sensitive to most of them. But unless it is known that someone has been exposed, it is still very difficult to take precautions against this kind of disease, since to be truly effective the treatment must start before the appearance of even the first symptoms.

In the United States, a human vaccine — free of any bacteria, live or dead — was developed in the 1960s. Available in limited quantities for general distribution, it is currently reserved for those in professions at direct risk (vets, research laboratory workers, military personnel, etc.).

Is anthrax a real threat?

Spread by means of aerosols, *Bacillus anthracis* spores can be an effective bacterial weapon thanks to their great resistance. In 1979, anthrax was at the heart of one of the most serious bacterial accidents ever, at the Sverdlovsk Biopreparat factory in the Urals, where it killed around a hundred people.

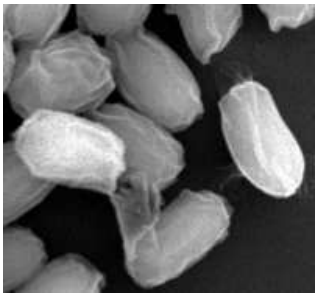


Fig.3 *Spores of Bacillus anthracis*

Despite this, we must not forget that *Bacillus anthracis* is currently and by far not the most dangerous bacterium around since it produces an illness which is not contagious in itself and that we know how to treat reasonably well. The great problem is to develop diagnostic tests that can detect the infection early enough. Without being truly reassuring, such a statement does fling the current situation into perspective. Indeed, it does seem that it is more panic than a true illness which is spreading like a bad cold…

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For further information

On the Web:

- Pasteur Institute (in French): www.pasteur.fr/pasteur/bioterrorisme.html#charbon
- Center for Disease Control (CDC): www.cdc.gov/ncidod/dbmd/diseaseinfo/anthrax_g.htm

In depth (and in English):

- Dossier prepared by the journal *Nature*: http://www.nature.com/nature/anthrax/

Illustrations:

- Fig.1, Source : http://www.anthrax.osd.mil/resource/images/images.asp
- Fig.3, Adaptation : http://er1.org/dataman.pl?c=lib&dir=docs/photos/Anthrax

At UniProtKB/Swiss-Prot:

- Protective antigen (AP), Bacillus anthracis : P13423
- Edema factor (FE), Bacillus anthracis : P40136
- Lethal factor (FL), Bacillus anthracis : P15917

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