6. Summary

The environmentally highly resistant *B. anthracis* spores cause natural infections in man and animals but have also been used as biological weapons. *B. anthracis* infection begins through intradermal inoculation, ingestion or inhalation of spores. Human pulmonary and gastric infections with *Bacillus anthracis* are almost always lethal, yet cutaneous infections usually remain localized and resolve spontaneously. Interestingly neutrophils are recruited to cutaneous but not other forms of *B. anthracis* infections, raising the possibility that neutrophils kill *B. anthracis*.

Neutrophils are the first cells of the innate immune system, which are recruited to a site of infection. Other cells of the innate immune system such as macrophages and dendritic cells have been implicated in the dissemination of *B. anthracis* and in the pathology of the infection, but not in its resolution. The role neutrophils play in *B. anthracis* infection has not been extensively studied.

B. anthracis is characterized by its two virulence plasmids encoding the two major virulence factors: the tripartite anthrax toxin and the poly-D-glutamate capsule. The toxin induces oedema and shock-like symptoms; the capsule was reported to have antiphagocytic properties.

In this study we infected human neutrophils with *B. anthracis* spores or vegetative bacteria expressing either of the two major virulence factors. As studied by electron microscopy, human neutrophils engulfed *B. anthracis* spores, which germinated intracellularly and were then killed. The killing of vegetative bacteria either expressing the toxin or the capsule was also examined. Both *B. anthracis* strains were phagocytosed and efficiently killed. Through inhibition of neutrophil phagocytosis with cytochalsin D we showed that the killing mechanism was of both intracellular and extracellular nature. Further, the killing of the vegetative cells was not due to reactive oxygen species but a result of the activity of a human neutrophil granule extract. This was elucidated by the use of an inhibitor of ROS production. We fractionated the neutrophil granule extract (hNGE) by high performance liquid chromatography with a combination of C4/ C4/ C18 reverse phase columns. α-defensins were identified by MALDI TOF TOF analysis as the component responsible for *B. anthracis* killing. α-defensins are constituents of the neutrophil azurophil granules, which have also been implicated in the neutralization of the anthrax toxin.

These data show that human neutrophils induce germination of *B. anthracis* spores and consequently kill the vegetative cells. This and the pathohistology of human

anthrax cases suggest that the timely recruitment of neutrophils in the human cutaneous form of the disease can control *B. anthracis* infections. This is supported by the fact that pulmonary infections in pigs and dogs show massive neutrophil infiltration, resulting in survival of the host.