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Review Article

War Against Anthrax

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A Current Topic Mini-Review

Introduction

Anthrax is a disease caused by the gram-positive bacterium *Bacillus anthracis.* The disease is usually confined to animals and is rare. Humans may be infected while handling disease-inflicted animals. *B. anthracis* has become a bane of defense establishments in various countries as, capitalizing on the formation of highly stable spores, it can be used as a potent biological warfare agent sprayed into the air in the form of a finely ground powder. Once inhaled, spores reach the lungs and become lethal. The outbreak of anthrax in Sverdlovsk, Russia, due to an accidental release of spores into the air, led to many deaths and shows the threat associated with the bacterium (1).

Human exposure to spores can go undetected for some time, during which the spores germinate inside macrophage cells (2). The population grows to critical-load level inside the body and, subsequently, secretes anthrax toxin into the system. Antibiotic treatment is effective if administered in time, in the initial stages of infection. Once a high enough concentration of the toxin has been released to cause irreversible damage to the body, the antibiotic ceases to be effective.

In that regard, ciprofloxacin has been found to be very effective against anthrax. However, excessive intake of the antibiotic can cause undesirable side effects, including a general depletion of the immune system.

Anthrax vaccine has been the sole source of preventing the disease. However, vaccination requires injection of a crude mixture of the toxin's constituent proteins in six doses. The situation poses the threat of a greater demand for the vaccine than its supply. Currently, the vaccination is restricted to military personnel.

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Discussion

Additional therapeutic measures need to be developed and implemented. In recent studies Bradley et al. (3) described the identification of the cellular receptor recognized by anthrax toxin and Lacy et al. (4) map an anthrax protective antigen binding site, which paves the way to develop effective inhibitors of anthrax toxin action. This brief report focuses on the recent developments towards the identification of molecules of therapeutic potential against anthrax.

Anthrax toxin is a three-protein exotoxin and is the major virulence factor associated with *B. anthracis.* The bacterial strains devoid of the toxin-producing activity are avirulent. The three constituent proteins are protective antigen (PA), lethal factor (LF) and edema factor (EF). PA binds to cell surface receptor and delivers LF and EF into the cell cytosol. EF is an adenylate cyclase and raises the intracellular cyclic adenosine monophosphate levels to non-physiological concentrations. LF is a metalloprotease and kills the cells by proteolytically modifying intracellular targets. PA + LF (termed lethal toxin) is the dominant virulence factor and kills cultured macrophage cells within 2 h and experimental rats within 90 min (5).

Although PA is a component of anthrax toxin system, it is non-toxic by itself. It is also the most immunogenic constituent of the toxin, a property that makes it an indispensable component of anthrax vaccine. After binding to the cell surface receptor, PA is cleaved by cellular protease furin. Cleavage results in the removal of N-terminal 20 kDa fragment and allows the 63 kDa receptor bound fragment (PA63) to bind LF/EF and oligomerize to a heptamer. The resulting oligomeric PA63-LF/EF complex undergoes endocytosis. Inside the endosomes, acidic pH results in membrane-insertion by the oligomeric PA63 ultimately resulting in the translocation of LF and EF into the cell cytosol where they exert their effects (6,7).

Extensive studies need to be done to block different steps of intoxication and develop potent inhibitors of toxin action. Different protein-protein

interactions, which are indispensable for anthrax toxin action, should be targeted. These include PA-receptor interaction, PA-PA interaction and PA-LF interaction. Bradley et al have reported the identification of cellular receptor for PA which is a type I membrane protein with an extracellular vonWillebrand factor A domain that binds to PA (3). Generating mutant CHO-K1 cell line that had deletions and frameshift mutations (introduced by adding ICR-191, a DNA-alkylating agent) and selecting cells resistant to anthrax toxin action identified the receptor. The defect was then genetically complemented to confirm the observation. This report has paved the way for development of inhibitors of toxin action by blocking PA-receptor interaction. New molecules can be designed that can specifically inhibit this interaction and ultimately block the toxin action. In fact, adding increasing amounts of the soluble extracellular domain of the receptor inhibited PA binding to the receptor[3]. In addition to developing inhibitors of the interaction, identification of the receptor will allow the detailed analysis of PA uptake and internalization.

Recent studies reported a novel therapeutic strategy that can not only act in concert with the PA-receptor inhibitor but can be utilized to fight other related diseases as well (8,9). The studies report identification of non-toxic mutant PA proteins that could assemble with wild-type PA to form inactive hetero-heptameric complex blocking the toxin action both in vitro and in vivo. Foundation has also been laid to inhibit PA-LF interaction. Collier and coworkers have reported identification of a polyvalent inhibitor of anthrax toxin action (10). The inhibitor, which is a peptide identified from a phage display library binds to oligomeric PA63 and inhibits its interaction with LF thereby neutralizing the anthrax toxin action. Developing inhibitors of the proteolytic activity of LF can further refine the approach.

Conclusion

Research in the past 2 years has contributed significantly to the development of new molecules against anthrax. Believably the day is not far off when molecules will be available that can neutralize any of the necessary steps of toxic action. For practical use, the most efficient therapeutic would be a marketable cocktail of multiple inhibitors, and antibiotics.

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