Biodefense: Trends and challenges in combating biological warfare agents

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Biodefense

Trends and challenges in combating biological warfare agents

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This special issue of *Virulence* is concerned with new trends and developments in biodefense research with emphasis in bacterial pathogens and toxins. Although the wealth of research approaches targeting this elite class of pathogens is increasing, there is an apparent gap in translating the accumulated information into effective medical countermeasures compliant with the current regulatory environment. This issue contains comprehensive reviews and covers a variety of innovative strategies for biosurveillance, molecular diagnostics, novel discovery, molecular tools, and alternative therapeutic interventions with focus on light-based platforms and immunotherapeutics. Special attention is placed on cornerstone biodefense pathogens such as *Bacillus anthracis*, *Burkolderia pseudomallei*, and *Francisella tularensis*.

Introduction

The possibility of biological warfare and bioterrorism has become an increasing concern to both military planners and civil defense authorities worldwide. Letters containing anthrax spores sent to destinations within the US in 2001 brought the sudden realization that bioterrorism is not merely a theoretical threat but a real and present danger. Although emergency preparedness and response capabilities exist throughout the Western hemisphere, the focus and availability of resources to deal with the actual and urgent health issues due to biological warfare has been diverted.[1] This new threat awareness motivated the research community to create at least two peer review journals in biodefense and also led to an extensive debate regarding how bioterrorism responses compare with those for pandemics and natural disasters.[2] The rigorous debate led to the mobilization of the scientific community, comprehensive research efforts, coordination of resources, and a first generation of medical countermeasures.[3]

A list of microorganisms/biological agents that pose the highest risk to national security and public health are classified as Category A select agents based on a set of considerations defined by the US Centers for Disease Control and generally approved by similar agencies worldwide. These biological agents share common alarming characteristics, as they are (1) highly morbid and lethal; (2) highly infectious or highly toxic; (3) amenable in wide distribution in an active form; (4) easily produced in bulk and easily stored until delivered; (5) reasonably hardy in the environment after distribution; and (6) amenable to genetic engineering to be resistant to antibiotics. The major challenge that is posed by these agents includes, among others, the treatment of infected/contaminated individuals. Antibiotic-resistant microorganisms are potentially near-ideal biological weapons.

Key Bacterial Biological Warfare Agents (BWAs)

These considerations can be met by a relatively small group of agents that can be divided into five classes of which representative examples include the bacterial species *Bacillus anthracis*, *Fransicella tularensis*, *Yersinia pestis*, *Brucella melitensis/abortus*, and *Burkholderia pseudomallei/mallei*. They are causative agents of fatal diseases such as anthrax, plague botulism, brucellosis tularemia, melioidosis, and glanders. There is a pressing need to elucidate their virulence and pathogenicity traits and translate the impact on the host by developing novel diagnostic and therapeutic tools and countermeasures. The chronological, geographical and epidemiological fingerprint of melioidosis exemplifies the need for comprehensive biodefense research efforts and their expected impact.[4] Melioidosis caused by *B. pseudomallei* is an equipotent biological threat with public health importance in endemic areas, particularly Thailand and northern Australia, with increasing frequency in other parts of the world. US servicemen have been exposed to the causative agent during the Vietnam War era where it was noted that helicopter crews seemed to have a high incidence of the disease. This and its long incubation period resulted in melioidosis acquiring the sobriquet "the Vietnamese time bomb", although remote exposures resulting in sporadic cases have continued to surface in the US.[5] Endemic melioidosis in Southeast Asia is on the rise, and in combination with the recent threat of gram-negative multidrug and pandrug antibiotic resistance, it was upgraded from a local time bomb in a global significant health concern.

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Over the last few years, the availability of genome sequences, metagenomic analyses, proteomic tools, and databases has increased geometrically for key biodefense bacterial pathogens.[6-11] The same applies for molecular microbiology and genetic manipulation techniques, such as the development of random insertional mutants for *B. pseudomallei*,[12] the allelic exchange in *F. tularensis*,[13] and the genome-wide screens for *B. anthracis*.[14] Finally, the reproducibility and regulatory compliance for relevant animal models is debatable, as it is reflected from comparative studies in *F. tularensis* and *Burkholderia* sp.[15] It is evident that the research activities centered around biodefense bacterial pathogens are active and vibrant, but there is a clear scientific gap, as well as a variety of unmet challenges for the development of effective diagnostic and therapeutic approaches and countermeasures.

Genomic-Wide Biosurveillance Approaches for Tracking Pathogen Virulence

The first paper by Willy Valdivia-Granda[16] correlates advances in genomic research with the unmet need to closely monitor and validate microbial biodefense threats. The lack of such systems compromises significantly any attempts to prioritize countermeasure needs and consequently hinders their development. The essential elements of a reliable biosurveillance system that supports a diverse community of users are described. A number of main obstacles and challenges for the development of this system are outlined. Despite the impressive advances in data mining capabilities, the real-time content analysis suffers from critical inconsistencies due to oversimplification attributed, for example, in the inability of social media to depict the factual reality. The inherent quality of the provided information is discussed and special notions are made for the counter effect of biased referencing and hash tags. This first article, more than any that follows in this issue, combines presentation of genomic scientific information with the social perspective and federal institutional responses to biological warfare agents (BWAs)

Broad Spectrum Biosensors for Diagnosis in Biodefense

A variety of different physicochemical instrumental techniques have been used for direct and indirect identification of bacteria as the basis for biosensor construction. The list includes: infrared and fluorescence spectroscopy, flow cytometry, chromatography, chemiluminescence techniques, as well as alternative enzyme- and immune-based assays. The idea of designing broad spectrum biosensors capable of identifying diverse organisms is quite appealing and the process is gradually transitioning from the bench top into the clinic. Metzgar et al.[17] discuss novel biosensor technologies for the identification of a variety of pathogens based on bioinformatic signature-matching processes. A clear distinction is made between technological approaches to detect individual organisms with those sensing microbial communities. Emphasis is placed on the impact of the latter approaches in biodefense applications, as well as the amenability for sensing unculturable or extremely hazardous microorganisms.

Comparative Analysis of PCR-Based Assays for the Detection of *B. anthracis* Chromosomal Fingerprints

The specific identification of *B. anthracis* and its differentiation from the closely related *Bacillus cereus* and *Bacillus thuringiensis* species remains a major diagnostic problem. The commercially available diagnostic kits targeting plasmid-markers have proved unable to distinguish *B. anthracis* from the non-*anthracis Bacillus* species. These kits usually harbor anthrax-specific virulence plasmids (pXO1 and pXO2) and *B. anthracis* strains that don't carry plasmids. Ågren et al.[18] provide a comprehensive analysis for both types of PCR-based assays for the detection of *B. anthracis*. They coupled experimental with in silico comparative methodologies for all the signature sequences available for the causative agent of anthrax. They comment on the sensitivity and the specificity of their observations: Out of the 35 PCR assays investigated, only 4 were 100% specific for the *B. anthracis* chromosome. Further testing of 6 assays, including the WHO recommended procedures, employing a collection of 90 *Bacillus* strains resulted in 3 adequately performing assays that share the same chromosomal marker location: the lambdaBa03 prophage region (PL3, BA5345, and BA5357).

The Staphylococcal Enterotoxins Pathogenicity, Host Responses, and Intervention Strategies

Krakauer and Stiles[19] provide a comprehensive review of a key virulence and pathogenicity determinant family of toxins—the staphylococcal enterotoxins (SEs). Although an aerosolized staphylococcal enterotoxin B (SEB) toxin weapon would not likely produce significant mortality, it could render 80% or more of exposed personnel ill and unable to function for weeks. Therefore, SEB has been historically considered as a potential BWA. These protein toxins target mainly the major histocompatibility complex class II on antigen-presenting cells and specific Vβ regions of T-cell receptors, resulting in potentially life-threatening stimulation of the immune system. The article summarizes models and molecular tools developed over the years to study the interaction of SEB with the host, as well as highlights progress on current therapeutic interventions with emphasis on vaccines.

Ribosome-Inactivating Proteins as Molecular Tools

Ribosome-inactivating proteins (RIPs) are protein synthesis inhibitors that act at the ribosome. They have been shown to exhibit RNA N-glycosidase activity and depurinate the 28S rRNA of the eukaryotic 60S ribosomal subunit. Members of the family include shiga and shiga-like toxins type I (trichosanthin and luffin) and type II (ricin, agglutinin, and abrin) RIPs. They have been considered as valuable molecular tools conjugated to monoclonal antibodies (immunotoxins to target cancer) or in the case of trichosanthin, targeting T cells and macrophages after an HIV-1 infection. Walsh et al.[20] report recent mechanistic findings regarding fungal ribotoxins and reviews the newly discovered *Burkholderia* lethal factor 1 (BLF1). BLF1 and fungal

ribotoxins don't possess RNA N-glycosidase activity despite their ability to block protein synthesis. This new evidence is the basis of a discussion for the classification and the exact functional and enzymatic capabilities of RIPs.

Small RNA-Mediated Regulation of Host–Pathogen Interactions

Small RNAs (sRNAs) constitute a large and heterogeneous regulator class implicated in bacterial gene expression. Bacterial sRNAs share functional similarity to eukaryotic microRNAs as global posttranscriptional regulators, through targeting multiple mRNAs by base pairing with multiple downstream target mRNAs to prevent translation.[21] Transcriptome sequencing reveals evidence for the presence of hundreds of different sRNAs in some bacterial species. The multiple regulatory roles in microbial physiology prioritizes them as a compelling class of targets in drug discovery. Harris et al.[22] emphasizes recent trends and molecular mechanistic facts and discoveries for the role of bacterial, viral, and human sRNAs in regulation of pathogen virulence and host immunity. The application of these findings in therapeutic approaches for biodefense bacterial pathogens is extensively discussed.

A Light-Based Therapeutic Platform for BWAs

Vatansever et al.[23] presents the challenges for opportunities employing light to develop a series of therapeutic applications for BWAs. Two major distinctions are made as far as it concerns UV and visible light: the germicidal effect of UV (UVC), a case is made for broad and selective microbicidal effect for bacteria and viruses and the photocatalytic, but also the potential effect in vaccine development for UVA. Visible light can destroy microorganisms alone—blue light is considering highly microbicidal with the ability to eradicate also bacterial spores. Finally, a special segment is devoted to photodynamic therapy (PDT). The concept of photodynamic inactivation in PDT requires microbial exposure to visible light energy that causes the excitation of exogenous photosensitizer molecules resulting in the production of singlet oxygen and other reactive oxygen species that react with intracellular components, and consequently produce cell inactivation. Besides the antimicrobial potential and range of PDT, the selectivity as well as the stimulation of the host immune system is discussed.

Adherence and Uptake of *Francisella* into Host Cells

Pulmonary exposure to *F. tularensis* results in the majority of the cases in severe lung pathology and a high mortality rate. During the early infection steps, the lack of induction of classical inflammatory mediators, including IL1-β and TNF-α has been observed. This led to the suggestion that *F. tularensis* probably is capable of evading detection by host innate immune surveillance and/or actively suppresses inflammation. The *Francisella* virulence factors and pathogenicity determinants, especially those that facilitate the interaction with the host, are largely uncharacterized and poorly understood. Very little is known regarding the invasion path and the host signaling cascades following invasion. Moretto and Mann[24] provide insights and updates regarding key adhesion and virulence factor genes in *Francisella* that are homologous to those involved in type IV pilus structure and assembly, including 6 genes encoding putative major pilin subunit proteins, present in the genome of the highly virulent Schu S4 strain. As attachment and internalization are essential in the pathogenicity process, this article offers insights for pathways that can be exploited in drug discovery.

Biofilms: An Advancement in Our Understanding of *Francisella* Species

Biofilm formation is a central virulence and antimicrobial resistance ingredient, as well as the molecular determinants and pathways related with biofilm production, in key nosocomial pathogens. The multiple roles of these microbial communities have been studied extensively with emphasis in the role of the capsule, carbohydrate quorum sensing, and two-component signaling systems. The ability of *Francisella* spp. to form biofilms as part of its pathogenesis arsenal is a recent and relatively unexplored area. Nevertheless, van Hoek[25] collected and evaluated an unparalleled data set from recent studies and make the case for the contribution of *Francisella* biofilms in environmental tolerance and survival. van Hoek interrogates major *Francisella* cell structural and functional elements, identifies their relation with the biofilm phenotype, and translates this information into virulence determination and clinical significance.

Particle Size and Pathogenicity in the Respiratory Tract

The respiratory track is a common niche and target for many select agent BWAs, especially for the 3 key pathogens that are extensively discussed in this issue. This is pivotal in the comprehensive understanding of pathogenicity, infectivity and host responses to aerosolized parthogens. Thomas[26] focuses on respiratory track infectivity and makes the interesting correlation between animal models currently under investigation and humans. Apart from the direct comparison and simulation, a significant portion of his analysis is centered around particle size, comparative infectivity between upper and lower respiratory track, and clinical manifestation attributed to either type of infection.

Immunotherapy for Tularemia

One appealing platform under investigation in a variety of pathological conditions is adjuvant immunotherapeutics. The selective stimulation of protective immune responses can serve as an alternative treatment option for bacterial infections. In order to elicit appropriate immune responses and to avoid undesirable inflammatory tissue damage, it is essential to identify

ligands and receptor pathways that specifically control protective responses at the infection site. The key biodefense pathogens are not exempt, but on the contrary they are intensively interrogated for the development of immunotherapeutic interventions. This approach has been recently explored. Skyberg[27] examines the utility of the platform in tularemia. He reviews the advances in the field for identifying compelling therapeutic targets in *F. tularensis* and discusses the challenges in translational research and target deployment against tularemia.

Conclusions and Future Perspectives

Research in BWAs and biodefense applications has been attracting attention, populating the literature and energizing military and civilian authorities and planners for the last decade. This excessive mobility is the result of an increase in the frequency of viable bioterrorism threats and the risk of intentional dissemination of BWAs. On the other hand, the biosafety security requirements for biodefense research as well as the nature of BWAs generates, by default, additional technical challenges in expediting successful countermeasures. These challenges share similarities and differences with the ones attributed to emerging multidrug resistant pathogens. The increasing antibiotic resistance over the past 50 years has transformed many dogmas in antimicrobial drug discovery leading to a gloomy prognosis of untreatable infections. The great era of antibiotic discovery and relative predictions for the "end of infectious diseases" are followed by the current consensus belief for the "end of the antibiotic era".[28] The response to biothreats appears more stochastic and comprehensive. The articles in this special issue highlight recently developed innovations and thematic biodefense priorities interventions to combat BWAs. They highlight sensitive diagnostic identification approaches and attractive therapeutic platforms under investigation that may prove helpful in developing new treatments or improve existing ones. Special thanks to all the authors for their excellent contributions and the publishing team of *Virulence* (Sara Sharpe and Eva Riedmann) for their help during the past 9 months in compiling this issue. We hope that readers will find in this issue new avenues of research in the fight against BWAs.

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