Proteomics for biodefense applications: progress and opportunities
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KEY WORDS: 2D SDS-PAGE, biodefense, biomarkers, ICA T, immunoproteomics, MALDI, SELDI

The increasing threat of bioterrorism and continued emergence of new infectious diseases has driven a major resurgence in biomedical research efforts to develop improved treatments, diagnostics and vaccines, as well as increase the fundamental understanding of the host immune response to infectious agents. The availability of multiple mass spectrometry platforms combined with multidimensional separation technologies and microbial genomic databases provides an unprecedented opportunity to develop these much-needed resources. An overview of current proteomic strategies applied to microbes and viruses considered potential bioterrorism agents is presented. The emerging area of immunoproteomics as applied to the development of new vaccine targets is also summarized. These powerful research approaches can generate a multitude of potential new protein targets; however, translating these proteomic discoveries to useful counter-bioterrorism products will require large collaborative research efforts across multiple basic science and clinical disciplines. A translational proteomic research paradigm illustrating this approach using influenza virus as an example is discussed.

While the potential large-scale use of biologic weapons has existed since the end of World War II, the anthrax letter attacks of 2001 in the USA ushered in a new and immediate need for improved countermeasures against bioterrorism agents. For the purposes of this review and the research descriptions herein, the term bioterrorism will be used as defined in the National Institute of Allergy and Infectious Diseases (NIAID) Strategic Plan for Biodefense Research as 'the use of microorganisms that cause human disease, or of toxins derived from them, to harm people or to elicit widespread fear or intimidation of society for political or ideologic goals. From a scientific and medical perspective, this form of terrorism is best seen as a variant of the general problem of emerging infectious diseases, the only difference being that increased virulence or spread into a susceptible population is a deliberate act of man rather than a consequence of natural evolution' [101]. The key to effectively counter these bioterrorism agents lies in the development of new rapid diagnostic tests, new vaccines and immunotherapies for prevention, and new drugs and biologics for treatments. As illustrated by the large numbers of potential bioterrorism agents included on the NIAID Category A–C Priority Pathogen list [102], a substantial investment in biomedical research on the properties of these pathogens, and the immune response to them, is required. In the USA alone, the 2004 NIAID biodefense research budget for biomedical research exceeded US$1.5 billion. The allocation of these types of resources to biomedical research offers the potential to further develop and utilize novel technologies. In this regard, few emerging technologies offer as much promise as those encompassed by the term proteomics, a biomedical research area that will increasingly provide new solutions and treatments against bioterrorism agents.

The goal of this review is to summarize the methodologies and experimental rationale of successful proteomic approaches that have already been accomplished in the context of...
In Table 1, illustrate a range of examples of what can be achieved applied to microbial systems. Overall, the types of studies listed have also been applied to microbial systems [12]. In practice, such as Fourier transform ion cyclotron resonance (FTICR)-MS utilized as front-end steps prior to MS. More recent MS platforms have been underrepresented in Table 1, but will likely be increasingly sophisticated affinity-based technologies such as ICAT are or some type of hybrid instrumentation. In contrast, more straightforward configurations, electrospray ion-trap mass spectrometers tryptic peptides determined using different MALDI instrumentation (SELDI)-TOF-MS are also increasingly being applied to low mass range protein display methods such as matrix-assisted laser desorption/ionization (MALDI) time-of-flight (TOF) mass spectrometry (MS) and surface-enhanced laser desorption/ionization (MALDI) mass spectrometry (MS). Comparative proteomic techniques such as isotope-coded affinity tags (ICATs) [8,9], as well as different multidimensional chromatography systems [10,11], are being utilized in the characterization of different MS platforms. The monitoring of proteomic differences in bacterial and viral forms, thus it is not surprising that most proteomic publications frequently utilized in the characterization of different MS platforms. Comparative bacterial proteome studies could be analyzed via another emerging technology, 2D differential in-gel fluorescence electrophoresis (DIGE) [17,18]. This involves differentially expressed protein samples for analysis as isotope-coded affinity tags (ICATs) [8,9], as well as different multidimensional chromatography systems [10,11]. Comparative bacterial proteome studies could be analyzed via another emerging technology, 2D differential in-gel fluorescence electrophoresis (DIGE) [17,18].

| Protein Target | Methodology | Applications |
|---------------|-------------|--------------|
| E. coli       | Proteomics  | Biodefense   |
|              |            | Infectious Disease |

In Table 1, a summary and representative list of different NIAID Priority Pathogens included in Table 1. One of the most common approaches used for the studies listed in Table 1 are proteome characterization studies for biodefense pathogens involving strains exhibiting diverse phenotypes including antibiotic resistance, altered degrees of infectivity and pathogenicity. The monitoring of proteomic differences in bacterial and viral infections of the review address this issue, including discussion of area in relation to other diseases such as cancer. The latter section is not intended to be exhaustive in scope, just indicative of more recent proteome characterization studies for multiple bacterial proteomes.

The monitoring of proteomic differences in bacterial and viral infections of the review address this issue, including discussion of area in relation to other diseases such as cancer. The latter section is not intended to be exhaustive in scope, just indicative of more recent proteome characterization studies for multiple bacterial proteomes. As illustrated most effectively with the human genome and its genome database for each organism being characterized for differential expression, these efforts. In this regard, there are a multitude of genomic base resources will greatly decrease the redundancy of sequencing efforts. As the organisms in Table 1 are priority pathogens, these continue to rapidly evolve as other bacterial genomes are continue to rapidly evolve as other bacterial genomes are continue to rapidly evolve as other bacterial genomes are continue to rapidly evolve as other bacterial genomes are continue to rapidly evolve as other bacterial genomes are continue to rapidly evolve as other bacterial genomes are continue to rapidly evolve as other bacterial genomes are continue to rapidly evolve as other bacterial genomes are.

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determinations of the biomarker candidates, necessitating the use of other strategies for this purpose. New generations of MALDI-TOF/TOF instrumentation are emerging that facilitate identification of prevalent peptide fragments less than 4000 mass-to-charge ratio \[21, 22\], thus increasing the types of proteomic profiling strategies that can be applied to biodefense pathogens. An example of applying MALDI to biodefense pathogen characterization is summarized in the following paragraphs, and an example of SELDI applications is presented in the next section.

Multiple MS-based studies have been reported for characterization of the unique sporulation and vegetative properties of Bacillus spp., particularly for Bacillus anthracis\[1, 4, 5, 11, 23–26\]. B. anthracis strains are found throughout the world; however, this wide geographic distribution is not reflective of great genetic diversity except for documented variable number tandem repeated sequences and single nucleotide polymorphisms used in phylogenetic relationships \[27, 28\]. While these genetic differences are important to further understand the pathogenesis of B. anthracis, proteomic methods can be applied to the identification of proteins that are differentially expressed under various culture conditions and during the course of infection. From a forensics and biodefense perspective, proteomic profiling approaches may be able to identify unique protein signatures that are specifically related to spore culture conditions as well as differences in virulence between strains of B. anthracis. In B. anthracis, the spore coats are surrounded by a hydrophobic, balloon-like glycoprotein shell termed the exosporium \[29\]. Multiple studies have described different protein components of the exosporium specifically, and these proteins include a collagen-like structural protein termed BclA, other integral membrane glycoproteins, and multiple embedded soluble proteins such as racemase and superoxide dismutase \[26, 30, 31\]. In the most comprehensive analysis thus far, over 750 different proteins in the endospores of B. anthracis Sterne were identified by multidimensional chromatographies and tandem MS sequencing methods \[11\]. Given the complexity and growth variability of the spore proteome and the many strains of B. anthracis, proteomic profiling of B. anthracis is particularly important for biodefense applications.
generated from each of the proteins present and their bacterial sources using standard peptides allowed rapid identification of the most abundant. Analysis were generated within 5 min. These sequenced peptides suitable for isolation and high-energy fragmentation approximately 4–125 kDa were obtained within 20 min, and bacterial sets of bacterial proteins with molecular masses of approximately 8–150 kDa were diagnostic for differences within this species [1].

Other experiments with an average mass resolving power of 4450 for proteins from different mass peptides/proteins could be determined when spore protein digests were assayed with weak cation ProteinChips (Ciphergen Biosystems) followed by SELDI-TOF spectra generation. No peak identities were determined, but a panel of four biomarker peaks could be correlated with weak cation ProteinChips (Ciphergen Biosystems) and influenza A (n = 203), pneumonia (n = 176), lung cancer (n = 176), and healthy controls (n = 659). Each sample was incubated with White Mountain Trisodium Citrate (WMTS, n = 29), and large non-SARS cohorts included samples indicative of fever after onset of fever (n = 1067) and non-SARS (n = 1067) cohorts [36].

Proteolytic digestion of small, acid-soluble spore proteins, the different method of on-probe solubilization and lyzed spore mixtures [25]. It was reported that using the TOF instrument to rapidly separate and identify mixtures of Bacillus spp. has proven to normally in a given system, this approach could be critical for countermeasures against these diverse anthrax strains.

Two recent reports evaluated the ability of a conventional MALDI-TOF study reported that differential profiles of low-molecular mass peptides/proteins could be determined when spore protein digests were assayed with weak cation ProteinChips (Ciphergen Biosystems). This modification of MALDI-TOF technology uses Protein-Chip arrays coated with a chemical surface (e.g., ionic, hydrophobic or metal) to affinity capture protein molecules from complex mixtures. Retained proteins are subsequently analyzed by TOF-MS. With the aid of SELDI software, a representative map depicting the mass-to-charge ratio, which correlates with infection, is generated. Without fractionation and removal of contaminants, as has been a consistent criticism of the proteomic profiling assay for the early detection of SARS infections, one of which was serum amyloid A (SAA). Subsequent SAA concentration determinations in 45 longitudinal serum samples found a good correlation with infection, indicating a novel marker, peroxiredoxin-II secreted by T-cells. In 2003, a new strain of coronavirus (CoV) was identified as the cause of severe acute respiratory syndrome (SARS), which infected over 8000 individuals and led to over 750 deaths worldwide. Five recent studies have applied proteomic profiling methods for analyzing serum or plasma cohorts collected from infected patients in an effort to identify early markers of this disease. As more genomic information becomes available for complex mixtures of microbes reflective of a given environment, it is likely that the differential markers reflect acute-phase responses; however, this does not preclude them as being useful to many hundreds of samples, population-specific protein expression profiles can be deduced that are characteristic of the group. The result is a fingerprint pattern unique for the designated group. The expression of certain proteins in the serum approach [37], or do the peaks reflect innate responses? Are these peaks only representative of acute-phase reactions in 45 longitudinal serum samples found a good correlation with infection, indicating a novel marker, peroxiredoxin-II secreted by T-cells. In 2003, a new strain of coronavirus (CoV) was identified as the cause of severe acute respiratory syndrome (SARS), which infected over 8000 individuals and led to over 750 deaths worldwide. Five recent studies have applied proteomic profiling methods for analyzing serum or plasma cohorts collected from infected patients in an effort to identify early markers of this disease. As more genomic information becomes available for complex mixtures of microbes reflective of a given environment, it is likely that the differential markers reflect acute-phase responses; however, this does not preclude them as being useful to many hundreds of samples, population-specific protein expression profiles can be deduced that are characteristic of the group. The result is a fingerprint pattern unique for the designated group. The expression of certain proteins in the serum approach [37], or do the peaks reflect innate responses? Are these peaks only representative of acute-phase reactions in 45 longitudinal serum samples found a good correlation with infection, indicating a novel marker, peroxiredoxin-II secreted by T-cells.
Proteomic studies have been reported for www.future-drugs.com. A comprehensive experimental approach should be emphasized.

Available for a particular antigen (e.g., genomic, proteomic, immunological), this study illustrates how combining all resources might be beneficial. The predictive computational screen of the anthrax genome identified this antigenic species. Of the eight proteins predicted, five had been identified in the computational analysis. This study was unique in that it described a combination of biological and computational resources.

Another example has been recently described for anthrax (B. anthracis). The chaperonins isolated from the integral membrane and whole-cell lysates were found to be antigenic. Another example is the use of sera from patients with tularemia to probe the immunoproteome. In this case, from patients suffering from tularemia, a smaller subset was selected for MS sequencing analysis.

Recognition of every potential epitope derived from the pathogen would need to be done in the identification of these low-mass sera biomarkers. Application of complex body fluid-derived antigens resulting from the SARS-CoV infection is being studied. Much work remains to be done in the identification of these low-mass serum antigens.

In vivo infection models can generate novel vaccine candidates. Immunoproteomics has been comprehensively reviewed and has the potential to be highly effective when coupled with other comprehensive analysis strategies as described in the preceding paragraph.

Bioengineering of the influenza virus to generate viral strains could facilitate these efforts. Another immunoproteomic approach focuses on characterization of the pathogen-derived peptides bound to major histocompatibility complexes (MHC) on antigen-presenting cells.

The human influenza virus is an ideal model for the comprehensive proteomic characterization of the pathogen. The authors' own proteomic efforts in biodefense research have been focused on characterizing the pathogen's proteome.

Bioengineering of the influenza virus to generate viral strains could be engineered to be drug resistant to current anti-influenza drugs. Introduction of a strain such as this could lead to the rapid spread of pathogenic strains that are resistant to current drugs.

The authors believe that the human influenza virus is a paradigm for the comprehensive proteomic characterization of pathogens. Why influenza virus as a paradigm? This is based on the need for improved influenza vaccine strategies and comprehensive proteomic analysis strategies as described in the preceding paragraph.

Influenza as a clinical biodefense paradigm system has the potential to be highly effective when coupled with other comprehensive analysis strategies as described in the preceding paragraph.

Influenza morbidity and mortality is realized primarily in older adults and caused by the immune response to influenza virus. Specifically, elevated levels of cytokines are associated with influenza symptoms, including fever and headache. In the USA alone, an estimated US$10 billion is spent annually due to the impact of influenza, and this cost will rise as the population of senior citizens rapidly expands.

The immune-naive human population is susceptible to influenza infection. An antibody response to vaccine antigens is not always effective, and influenza vaccines are not always cost effective, but far from perfect. The authors believe that the human influenza virus is an ideal model for the comprehensive proteomic characterization of pathogens.

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underexpressed in day 4 sera from all six proteins were significantly (p < 0.05) over- or

at day 4 compared with day 0. On all SELDI profiles were observed, particularly days 1, 2, 4, 7 and 14 post vaccination.

subject immediately before (day 0) and on

and nas al sw ab w ere obtained from each virus FluMist vaccine intranasally. Serum age) were recruited, and received the live healthy young volunteers (21–30 years of age) were vaccinated subjects are presented in the next section to illus-

trate how effective this approach could be for clinical biodefense studies. T wo examples from a ideal system for applying current and emerging proteomic methodologies, cell line  and animal infection models. Clinically , research, including well-defined viral stock preparation meth-

ologies, cell line  and animal infection models are a vailable for influ enza agents are also available. Cumulatively , obtaining a statistically defined antibody detection assays and clinically useful antiviral agents are also available. Cumulatively , obtaining a statistically defined antibody detection assays and clinically useful antiviral...
studies related to HIV are included, and this should be an embarrassingly sparse body of literature in this field, even if application of proteomics to viral pathogens. There is an biodefense applications.

better methods and instrumentation will quickly emerge for biomedical research, there is no reason not to expect that even these statements are based on currently proteomic technolo-

animal and plant diseases, and different environmental systems. developed for biodefense applications could be enormous when eventual collateral benefits of the proteomic methodologies impact many other areas of human health research, and the tation of new treatment targets. The mechanistic and func-

genes, particular anthrax. A clear convergence of proteomic, advanced proteomic resources for application to bacterial path-

progress in applying and developing the most innovative and well as reasons for great concern. There has been excellent in the context of utilizing proteomics and related resources for expert opinion & five-year view.

other applications.

for vaccine efficacy, treatment response, disease progression and sent a largely uncharacterized reservoir of potential biomarkers also for any pathogen should be considered as these fluids repre-

collection of body fluids during vaccination (or treatment) tri-

Figure 2. Representative SELDI spectra of nasal swab extract proteins post from a FluMist PLU NC: Palate, lung and nasal epithelial clone; SE LD I: Surface-enhanced laser desorption/ionization. IMAC-Cu: Copper-coated immobilized metal ion affinity chromatograph; MW: Molecular weight; polyacrylamide gel electrophoresis indicated two unknown membrane proteins and PLUN C. spectrometry sequence identification of the three main peaks from day 1 following sodium dodecyl sulfate peak intensities. The box indicates a different peak pattern between the two samples. Tandem mass solution and 1 µl of fluid was loaded. The indicated arrows highlight the difference in the scale of the vaccine, day 0 versus day 1.
to biodefense studies. Specific to cancer-related studies, and will be equally applicable at pharmaceutical levels, is highly encouraging. These issues are not readily addressable, and are actively being pursued across multiple clinical, academic, biotechnology and biopharmaceutical data analysis tools. The fact that all of these issues are cumulatively highlighted several areas that have been performed for cancer diagnostics, and these cumulative experiences have highlighted several areas that have already been learned from applying these technologies to developing cancer diagnostics.

For the more clinical application of proteomics, typified by SELDI and MALDI analysis of blood fluids, there are numerous applications of isotope-coded affinity tag methods and Fourier transform ion cyclotron resonance MS for the discovery of new therapeutic and vaccine targets. Characterizing the proteomes of bacterial pathogens, as well as the host response to viral and bacterial infections.

Opportunity to fully characterize the proteomes of individual pathogens. As appropriate genomic databases, there is an unprecedented access to FTICR-MS instrumentation increases, coupled with miniaturized MALDI time-of-flight MS and surface-enhanced laser desorption/ionization time-of-flight MS is a largely untapped approach.

Comparative proteomics of bacterial proteomes will further expand the dynamic range of bacterial proteomes and greatly facilitate the characterization of bacterial proteomes and improved 2D sodium dodecyl sulfate PAGE methodologies, such as differential gel electrophoresis, will further refine and extend the information gathered from the approach.

Ilustrates the ability of proteomics to generate comprehensive and comparative subproteome information. 2D gel electrophoresis (PAGE) with mass spectrometry (MS) identification is still the most accessible approach for characterizing the proteomes of bacterial pathogens, and improved 2D sodium dodecyl sulfate PAGE methodologies, such as differential gel electrophoresis, will further refine and extend the information gathered from the approach.

For the more clinical application of proteomics, typified by SELDI and MALDI analysis of blood fluids, there are numerous applications of isotope-coded affinity tag methods and Fourier transform ion cyclotron resonance MS for the discovery of new therapeutic and vaccine targets.

Increased applications of isotope-coded affinity tag methods and Fourier transform ion cyclotron resonance MS for the discovery of new therapeutic and vaccine targets.
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