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Commentary

Antibody gene-based prophylaxis and therapy for biodefence

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Abstract

The threat from the use of biowarfare (BW)/bioterrorism (BT) agents is now more likely than ever. Antibodies, which are naturally produced molecules with high specificity and affinity, play an important role in immune defence by recognizing and eliminating invading microbial pathogens or neutralizing toxins. Passive antibody administration is an effective means of conferring immediate immunity to a susceptible host for post-exposure prophylaxis or therapy of BW/BT agent-mediated diseases, but the immunity would not last long and antibody production is a lengthy, labor intensive, and expensive process. An alternative approach is to take advantage of the body's natural ability to express transgenes to produce passive antibodies. This approach can be achieved by the in vivo delivery of genes encoding BW/BT agent-specific antibodies for biodefence applications. It is also possible to design antibody fragments to be expressed inside a cell via antibody gene delivery for combating intracellular BW/BT agents and toxins, which natural antibodies cannot reach. Animal studies have shown that the expressed antibodies can be detected as early as day 3, reaches peak levels at day 7, and maintains therapeutic levels in serum for more than seven months after a single administration via antibody gene delivery. Therefore, antibody gene delivery in vivo might be a new approach for post-exposure prophylaxis or therapy and for pre-exposure prophylaxis (vaccination) of BW/BT agent-mediated diseases although there are still some problems to be overcome before this new approach can actually be used in humans.

Biowarfare (BW)/Bioterrorism (BT) Agent Threats

BW/BT agents are microbial pathogens or toxins, which are deliberately used to kill, injure and/or incapacitate people. They share one or more of these characteristics: virulence, rapid onset and dissemination of diseases, and antibiotic resistance. BW/BT agents are considered as weapons of mass destruction. Although the list of potential BW/BT agents suggested by US Centers for Disease Control and Prevention (including viruses, bacteria, rickettsiae and toxins) is short, BW/BT agents, if disseminated properly, could cause serious harm to society.

The intentional release of BW agents is not a new concept, and has been reported throughout history.[1] Since the 1980s, there have been incidents where BT agents have been used. As recently as the beginning of this century, there were at least two well publicized cases. In the fall of 2001, letters containing *B. anthracis* spores were delivered by mail to US commercial media and government offices in New York, Washington DC, and Florida.[2] A total of 22 confirmed or suspected cases were reported. Eleven cases of inhalational anthrax resulted in five deaths, while 11 cases of cutaneous anthrax (seven confirmed, four suspected) were also reported. In February of 2004, three Senate office buildings were closed after the deadly toxin, ricin, was found in the mailroom that serves Senate Majority Leader Bill Frist's Office.[3] The threat from the use of BW/BT agents is now more likely than ever and is one of the reasons why research on anti-BW/BT agent countermeasures has been increased and highly focused.

Antibodies Against BW/BT Agents

Antibodies, which are naturally produced molecules with high specificity and affinity in the immune system, recognize and eliminate evading pathogens by binding to surface antigens. The antibody molecule is composed of four polypeptide chains: two identical light chains and two identical heavy chains joined by disulfide bridges. Each chain has a variable portion that is unique to each antibody and is the active portion of the molecule that binds to the specific antigens on pathogens. Antibodies can function against invading pathogens by two principal actions. The first is direct interaction with the pathogen including direct antimicrobial effects, toxin neutralization, viral neutralization, and interference with microbial attachment. The second is indirect engagement of immune responses through activation of crystal fragment receptors including antibody-directed cellular cytotoxicity, opsonization and phagocyte-mediated phagocytosis, and complement-activated cytolysis. Treatment against BW/BT agents by administrations of therapeutic antibodies into the body, called passive antibody therapy, has substantial advantages over other therapeutic approaches,

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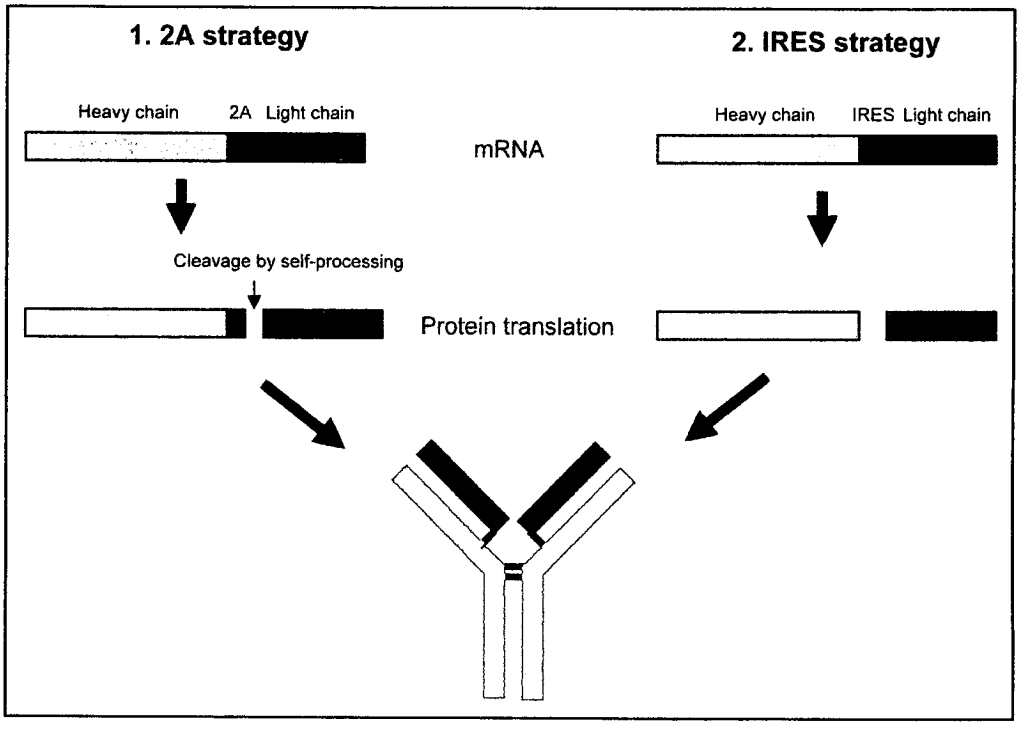

Figure 1. Two strategies for expression of a full-length mAb from a single open reading frame driven by a single promoter.

including low toxicity and high specific activity, and a more immediate effect compared with vaccines and antibiotics. Historically, there have not been any approaches available for the neutralization of toxins apart from the administration of antibodies. Antibodies can be used against most of the potential BW/BT agents such as *Bacillus anthracis,*[4,5] *Clostridium botulinum,*[6,7] *Yersinia pestis,*[8,9] *Francisella tularensis,*[10-12] Variola major virus,[13,14] Ebola virus,[15-18] Venezuelan equine encephalitis virus (VEEV),[19,20] West Nile virus,[21,22] Ricin,[23,24] and Staphylococcal enterotoxin B.[25]

Passive Antibody Therapy Against Infectious Diseases

Passive antibody therapy against infectious diseases in humans was first developed a century ago.[26,27] Since the therapeutic antibodies were obtained from human donors or large animals as immune sera, passive antibody therapy was referred as to serum therapy. At that time, serum therapy was used to treat some infections caused by microorganisms which are currently classified as potential BW/BT agents such as *B. anthracis*, Brucella species and Variola major virus. Despite unquestioned efficacy in the fight against infectious diseases, serum therapy suffered from a number of drawbacks, including high cost, a cumbersome process, batch-to-batch variation, low content of specific antibodies, and infectious risk of plasma-derived products. Consequently, serum therapy was largely abandoned in the 1940s when antibiotics were introduced into clinical practice. The development of monoclonal antibodies by mouse hybridoma technology in the late 1970s opened a new era in passive antibody therapy, since monoclonal antibodies are monospecific, homogeneous, and reproducible.[28] However, it was soon found that these monoclonal antibodies had serious disadvantages as therapeutic agents in humans. Murine monoclonal antibodies induce human anti-mouse antibody (HAMA) responses.[29] Repeat administration of these antibodies may result in rapid clearance of the murine monoclonal antibodies and anaphylaxis, which can sometimes be fatal. Using hybridoma methods to immortalize human B-cells would mitigate these problems, but the absence of a suitable fusion partner for human B cells and other technical issues have made methods that rely on human B-cell immortalization somewhat problematic. To overcome this hurdle, humanization of murine monoclonal antibodies can be implemented. Modern alternative strategies now even allow development of fully human antibodies directly from phage-display libraries of human antibody fragments.[30] Another approach is to use mice that are transgenic for the human immunoglobulin locus.[31] Immunization of such a transgenic mouse leads to the development of human antibodies, from which hybridomas that produce human antibodies can be generated.

To date, at least eighteen monoclonal antibodies have already been approved by the US Food and Drug Administration for therapeutic use in patients.[32] More than 150 therapeutic antibody-based clinical trials are currently ongoing for treating a variety of diseases.

Production of human antibodies or humanized antibodies is lengthy, laborious, and costly, especially using large-scale mammalian cell culture. Another drawback is antibody purification. Current technologies can provide sufficiently pure but not 100% pure antibodies for human therapy. Impurities from the purified antibodies can be toxic to humans. In addition, passive antibody therapies are usually administered intravenously via hour-long infusions, which require a specific hospital environment and are often associated with mild to severe side effects including vomiting, migraines, anemia, and thrombocytopenia. This approach would be impractical when large populations are exposed. Furthermore, the efficacy of passive

antibody therapy is short-lived due to the short antibody half life (around two weeks).[33] Efficiency would also be reduced if the antibody is administered after the onset of symptoms of microbial infection because antibodies cannot enter cells to take effect when microbial pathogens have already entered the cells. Technological advances in gene delivery make it possible to deliver an antibody gene into a person and have the human body itself produce therapeutic antibodies from delivered DNA vectors carrying antibody genes so as to overcome the drawbacks of the passive antibody therapy.

Antibody Gene Delivery In Vivo

There are two important issues, which need to be considered about antibody gene delivery in vivo before this approach can be applied in biodefence. The first question is the serum level of expressed antibodies after gene delivery in vivo. Would sufficient antibodies be expressed in vivo to exert a therapeutic effect? Recombinant adenoviruses are one of the preferred viral vectors used in gene therapy, since these vectors produce a high yield of recombinant proteins.[34] Recently, Fang et al.[35] achieved a remarkably high level (>1,000 μg/ml) and long-term expression (>140 days) of a monoclonal antibody against anti-vascular endothelial growth factor receptor 2 in mice after one injection of an antibody gene and showed in vivo therapeutic efficacy against two tumor cell lines. Jiang et al.[36] reported a single injection of recombinant adenoviral vector encoding an anti-tumor antibody in mice resulted in not only 40 μg/ml serum antibody level for at least four weeks, but also significant tumor elimination in the ovarian cancer SKOV-3-inoculated nude mice. Both studies indicate that it is indeed possible to express antibodies at a sufficient level in vivo for therapeutic efficacy using the antibody gene delivery approach.

The second issue that should be taken into account is the biochemical nature of antibodies. Antibodies are large molecules composed of two chains that need to be assembled into a four subunit structure to exert their functions. Expression of two chains in two vectors in vivo could result in an imbalance in heavy and light chain production. Extra chain production might be toxic to the antibody-expressing cells. At present, it is a challenge to produce heavy and light chains in one vector. A foot-and-mouth-disease virus-derived 2A self-processing sequence could be introduced between heavy and light chain DNA sequences to express a full-length antibody from a single open reading frame driven by a single promoter in an adenoviral vector.[35] The 2A oligopeptide sequence can undergo self-cleavage to generate the antibody heavy chain and light chain after translation (Fig. 1). Another approach is to insert an internal ribosomal entry site (IRES) between the antibody heavy chain and the light chain genes in a bicistronic vector under a single promoter.[36] Transcription from this bicistronic vector produces a single mRNA molecule encoding both heavy and light chains. IRES enables the ribosome to bind to the initiation site of the light chain. Thus, as showed in Figure 1, the heavy chain and light chain are translated separately from the same mRNA molecule and expression levels of both chains are thereby paired.

Feasibility of Antibody Gene-Based Pre-Exposure Prophylaxis (Vaccination) Against BW/BT Agent-Mediated Diseases

Vaccines are one of the greatest revolutions in modern medicine. The development of vaccines is also one of the most important medical countermeasures against BW/BT agents. Although pre-exposure vaccination against BW/BT agents for the general population does not appear as the best solution in absence of a clearly identified BW/BT threat, the first-line responders such as military, police, firefighters, and emergency medical professionals should be vaccinated against BW/BT agents. The benefits of vaccines to prevent BW/BT agent-mediated diseases are countless. However, the development of vaccines against BW/BT agents suffers from some drawbacks. Not all vaccine recipients mount a protective response, even after receiving the recommended immunization schedule. For example, the TC-83 vaccine against VEEV does not elicit an antibody response in approximately 18% of vaccine recipients.[37] In addition, immuno-compromised persons are often unable to generate effective immune response to vaccination.[26] Moreover, some anti-BW/BT agent vaccines are live attenuated vaccines. The risk of these vaccine strains to revert to virulent strains is cause for concern. Vaccines for most potential BW/BT agents are still in various stages of research and development with only a few licensed vaccines against BW/BT agents currently available.

In vivo delivery of antibody genes for pre-exposure prophylaxis is an alternative to the vaccines. The most attractive feature is that expressed antibodies will function regardless of whether the host has a fully functional immune system.

Lorenzen et al.[38] reported that a single chain variable fragment (ScFv) recombinant antibody was expressed in vivo to protect fish against viral pathogens. They constructed an eukaryotic expression plasmid encoding a ScFv neutralizing antibody to the fish viral hemorrhagic septicemia virus (VHSV). They then showed that rainbow trout fingerlings injected intramuscularly with the recombinant plasmid were protected against VHSV challenge. Kasuya et al.[39] demonstrated that mice that had received anti-anthrax protective antigen (PA) ScFv gene delivery in an adenovector were challenged with *B. anthracis* lethal toxin three days later, exhibited a significant survival advantage (100%). Most importantly, the therapeutic level of expressed antibodies in vivo after one administration remained for at least seven months.[40]

Feasibility of Antibody Gene-Based Post-Exposure Prophylaxis or Therapy of BW/BT Agent-Mediated Diseases

For prophylaxis or therapeutic purpose after exposure to a bioterrorist attack, a rapid buildup of antibodies in the body is required. How fast can expressed antibodies reach a therapeutic level through antibody gene delivery? A recent study showed that an expressed antibody could be detectable in vivo as early as day 3 and reached a peak level >160 μg/ml in serum at day 7 after one administration.[36] Another study demonstrated that mice that were transfected with an anti-PA antibody gene as early as up to 1 day pre-challenge had a significant protective response against anthrax lethal toxin challenge.[39] This result was more rapid than expected.

The most attractive advantage of antibody gene delivery for post-exposure prophylaxis or therapy of BW/BT agent-mediated diseases is that antibodies can stay inside of cells after expression and combat intracellular microbial pathogens and toxins. These antibodies are referred as to intrabodies (antibodies for intracellular applications). The major activity of intrabodies is to inhibit the function of intracellular antigens. Therefore, the antigen-binding site of antibodies is sufficient for this activity and ScFvs represent

the recombinant minimal antigen-binding fragments of antibodies. Thus, ScFvs are the most commonly used format for intrabodies expressed intracellularly.

The potential of intrabodies to neutralize the function of intracellular proteins has been demonstrated in clinical trials for treatment of various infectious diseases, particularly HIV. Intrabodies directed against several HIV proteins such as the viral coat proteins gp120[41] or gp41,[42] and proteins involved in transcription,[43] replication[44] or integration[45] of the viral DNA into the host genome, have shown efficient inhibition of HIV-1 production in host cells. Intrabodies have also been described for the treatment of various other viral infections including tick-borne flavivirus with a neutralizing intrabody directed against the envelope protein[46] and hepatitis C virus with an intrabody recognizing the non-structural 3 protein.[47] Although only few intrabody approaches have been described for the therapy of bacterial infections, intrabodies should have the potential to treat these diseases.

Conclusions

Advances in gene delivery approaches have demonstrated the possibility of in vivo production of anti-BT/BW agent antibodies encoded by delivered transgenes for biodefence applications. Optimization of in vivo antibody production, validation of the efficacy against BT/BW agents and safety studies are still required before any human application can be undertaken.

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