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Technical Transformation of Biodefense Vaccines

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Abstract

Biodefense vaccines are developed against a diverse group of pathogens. Vaccines were developed for some of these pathogens a long time ago but they are facing new challenges to move beyond the old manufacturing technologies. New vaccines to be developed against other pathogens have to determine whether to follow traditional vaccination strategies or to seek new approaches. Advances in basic immunology and recombinant DNA technology have fundamentally transformed the process of formulating a vaccine concept, optimizing protective antigens, and selecting the most effective vaccine delivery approach for candidate biodefense vaccines.

Biodefense vaccines have been developed against a diverse group of pathogens. In this Vaccine supplement issue of “Biodefense Vaccines”, reviews are included to describe seven bacterial vaccines (*Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Clostridium botulinum*, *Clostridium perfringens*, *Brucella*, and *Rickettsia*), five viral vaccines (smallpox, Venezuelan equine encephalitis, influenza, hantavirus, and Rift Valley fever) and two additional viral vaccines against pathogens that cause diseases in animals (foot and mouth disease and blue tongue). For several pathogens, effective vaccines were developed in the past, such as those for smallpox, *B. anthracis*, *Y. pestis*, *F. tularensis*, and influenza. The discovery of vaccination to prevent smallpox in 1796 by Edward Jenner actually marked the start of vaccinology [1]. The smallpox vaccine was so successful that it eventually eradicated this disease completely from the human population. However, when vaccines need to be produced for purposes of biodefense in the 21st century, the challenges appear greater than we would have expected. While governments of developed countries are willing to allocate many billions of dollars for the production and stockpiling of biodefense vaccines to protect the public from a potential bioterrorism attack, there appears to be a bottleneck for actual production of these vaccines, as evidenced by the vaccines on the government's procurement list that still have yet to be produced.

The reasons for drastic changes over the past 200 years to produce the same smallpox vaccines deserve more review; however, one of the key factors is the continuous and significant change on the social attitude and government regulatory controls regarding the safety standards for vaccines, as part of the broad medical products. Modern concepts of good manufacturing practice (GMP) basically disqualify many vaccines developed and produced during pre-GMP time as human vaccines. “Efficacy first” has become “safety

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first” during regulatory review of candidate vaccines. At the same time, the definition of safety and society's tolerance towards the risks associated with vaccines are also evolving. Mortality, which was rare even with the old vaccines, can no longer be viewed as the only valid safety standard. Rare vascular [2, 3] or autoimmune findings [4] can halt a vaccine clinical trial along its development pathway. Even diseases of low frequencies and unknown linkages to vaccines can lead to the complete withdrawal of a vaccine, even after its licensure [5]. While not all of these examples are directly related to the development of vaccines for purposes of biodefense, the development of biodefense vaccines also faces the same regulatory environment. It is important to stress that the intention and practice of more stringent regulatory review are a reflection of society's demands and, as a result, it is not fair to simply blame a regulatory agency or any administrations of a particular government for making the development of vaccines more difficult. Rather, it falls upon researchers in the field of vaccinology to advance vaccine technology to meet increasingly higher regulatory requirements in addition to the need to identify immunogens and exploit them for vaccine development.

Significant progress in immunology has provided vaccinologists with increased knowledge of human vaccines. At the same time, deeper knowledge in the theory and practices of modern-day immunology has created a discord between empirical and rational approaches for vaccine development. Frequently, the empirical approach will be labeled as “not hypothesis driven”. The scientific community is no longer satisfied with a candidate vaccine unless detailed and highly specific hypotheses can be provided on its possible mechanisms of protection and efficacy. As a result, the development of new generation biodefense vaccines requires both immunogenicity and mechanistic studies to provide more complete description and justification for the candidate vaccines, which is a significant change from past-generation vaccines.

No doubt, the development and production of biodefense vaccines in the 21st century is experiencing a fundamental technology transformation to meet new challenges. The following sections provide a brief overview of this movement (Table 1).

Changes in manufacturing processes

Anthrax bioterrorism attack that ensued in the U.S. shortly after September 11, 2001 was the key turning point in the history of biodefense vaccine development. Suddenly, the need for biodefense vaccines became eminent and moved from the traditional military market (i.e. protecting soldiers from biological weapons) to one incorporating both military and public use. However, many problems associated with the production of early generations of biodefense vaccines were also exposed. Vaccines for smallpox and anthrax provided two typical examples.

The traditional smallpox vaccines were produced by infecting the skin of calves or other large animals. The infected areas were then scraped to collect the lymph exudate, and live attenuated virus was purified and stabilized as human vaccines [1]. Such production continued until the 1970-1980s. In the early 2000's, when a new stock of smallpox vaccine was needed, it was clear that the antiquated and primitive manufacturing process for this vaccine was no longer acceptable for modern-day safety standards.

For the available anthrax vaccines, avirulent bacterial culture extracts are produced and the secreted protective antigen (PA) formed the basic component of Anthrax Vaccine Absorbed (AVA). AVA was licensed in 1970s but its release for routine use was only approved after the manufacturers provided supplemental information related to the manufacturing process in addition to a change in the label and package insert in 2002. As noted by the US General Accounting Office in 2002 during its review of existing anthrax vaccines, “general public

health vaccines are produced according to cGMP and are in constant, routine use worldwide. This use permits real time monitoring of whether the vaccines are performing properly. In contrast, biodefense vaccines have no such ongoing reality check because of the absence of natural disease and relatively limited use” [6].

Since then, biodefense vaccines no longer receive special treatment. The same stringent FDA review, based on cGMP requirements, is now applied to vaccines developed for the purpose of biodefense as is required for other, more routine human vaccines. A new version of the smallpox vaccine was produced by using the cell culture method, a major technical advance from its original production in live animals. This new smallpox vaccine, ACAM2000, was licensed in 2007 and later selected for national stockpile in the U.S [7].

However, other biodefense vaccines have not been able to make similar rate of progress in the manufacturing process (Table 2). A recombinant PA protein-based anthrax vaccine has not advanced fast enough to replace AVA, although, the PA protein vaccine was initially considered a relatively easy vaccine candidate [8]. Many biodefense vaccines are currently under development at various smaller biotechnology companies that lack significant experience in vaccine development and manufacturing when compared to the larger pharmaceutical companies. Some of them rely on limited product pipeline and usually cannot invest significantly in more complicated manufacturing process. The big pharmaceutical companies did not appear to be interested in biodefense vaccines considering that the only potential customers for biodefense vaccines are government agencies who can revise procurement policies or require new paperwork at any time with limited interest in compensating the extra cost associated with early research and development effort of biodefense vaccines.

Additional features of biodefense vaccines have made the process of producing biodefense vaccines even more challenging. The requirement of an extended shelf-life, for stockpiling reasons, is one such complication. Ideally, a biodefense vaccine should be expected to preserve the high levels of efficacy after being stockpiled for at least 3-5 years and possibly longer. Due to the high cost associated with stockpiling of vaccines, benefits of producing biodefense vaccines that can withstand extended periods of shelving are evident. This is a major challenge to the current vaccine manufacturing process since most routine vaccines do not need to be stored for extended periods of time, and, as a result, are more often produced for use in the near future due to cost and quality control issues.

Another issue underlying the development of biodefense vaccines is that the main pathway of transmission for several lethal bioterrorism pathogens is considered to be aerosol-based. Although there are new aerosol technologies to deliver vaccines, they would require a redesigning of the manufacturing process to formulate vaccines to match the downstream aerosol delivery equipment as well as the viability of the product due to humidity and temperature [9].

Adjuvant is also receiving significant attention in biodefense vaccines because it has become clear that not only the subunit vaccines, but also the inactivated vaccines, will need adjuvants to be part of a highly immunogenic vaccine formulation [10]. The complex nature of adjuvant-vaccine formulation presents an additional challenge in biodefense vaccine development (see below).

Reverse genetics approach used in influenza vaccine production improved a key step in the manufacturing of influenza vaccines, i.e., the generation of seed strain viruses that are used to produce both inactivated and live attenuated influenza vaccines [11, 12]. Reverse genetics can not only speed up the generation of the typical reassortant seed strains to seasonal and low pathogenic influenza viruses but also produce a safe seed strain for the highly

pathogenic H5 or H7 strains because the molecular features of HA antigen which confers the high virulence of these viruses can be removed during the process of reverse genetics, improving the safety of manufacturing vaccines for highly pathogenic influenza viruses.

Correlates of immune protection

Traditionally, it has been postulated that vaccines are effects through the induction of protective antibodies in the host. In recent years, with the availability of more sophisticated biomarker assays that were initially developed for routine human vaccines, the roles of T cell immune responses have been better recognized in biodefense vaccine studies.

One surprising finding came from plague vaccine studies. Smiley and colleagues showed that B cell-deficient mice vaccinated with live attenuated *Y. Pestis* were protected against plague while depleting T cells at the time of challenge abrogated protection and transferring vaccine-primed T cells to naïve mice provided protection [13]. These results established that cellular immunity mediated by vaccine-primed T cells can indeed protect against plague.

By using a mouse model, Berzofsky's group demonstrated that although antibody was essential to protect against disease by smallpox vaccines, T cells were necessary and sufficient for survival and recovery in the absence of protective antibodies [14]. Furthermore, biodefense vaccine-specific T cell immune responses can be present in immunized human populations for a long period. Demkowicz et al. showed that long-lived vaccinia virus-specific memory cytotoxic T cells were present in adults who had been immunized against smallpox as children. In people who had been immunized 35-50 years earlier, significant CD8+ and CD4+ T cell responses to vaccinia virus were detected after *in vitro* stimulation while no such responses were detected in young adults with no history of immunization against smallpox [15].

The above studies challenge us to re-evaluate the traditional way of thinking on how biodefense vaccines may work, which is important since some previous generation biodefense vaccines have been widely used for many years. Identification of the role of T cell immune responses with biodefense vaccines raised the question of what the correlates of protection for candidate biodefense vaccines may be. In many cases, there is no gold standard to make such judgment. It becomes even more complicated that for some biodefense vaccines, both antibody and T cell immune responses may be important for protection. At this point, it is unclear whether any successful biodefense vaccine will require strong responses for both arms of immune system. A key conceptual challenge is encountered when one takes into account that protective antibodies may be effective at the time of pathogen invasion, or an anamnestic response can be quickly induced to produce high level antibodies to eliminate or limit the infection, while it may take days to produce an antigen-specific T cell immune response, which may not be soon enough to prevent the pathogen from establishing an infection. In general, T cell responses are mainly effective against infected cells but not cell-free pathogens. The answer to these questions may depend upon the individual pathogen or biodefense vaccine in question, however, issues surrounding immune correlates of protection should be the first critical step to consider in the design of biodefense vaccines.

A related issue is the development of validated biomarker assays using good laboratory practice (GLP) and appropriate standards to measure the particular component of immune responses as the correlates of protection. In the case of cell mediated immunity, significant progress in recent years has been made in developing more quantitative and reproducible assays such as ELISPOT and intracellular cytokine staining with human peripheral PBMCs. Novel antibody assays are also under rapid development. New assays for detecting protective antibodies, such as the use pseudotyped viruses, will provide functional

measurements in addition to traditional ELISA-based antibody assays. B cell ELISPOT may provide more information on the memory B cell status. Highly sensitive and quantitative solid phase-based assays can now detect multiple antigen specific antibodies at even very low levels. The combination of these biomarker assays will contribute to the identification of correlates of protection for future generations of biodefense vaccines.

Subunit protein or gene-based vaccination approaches

Traditional biodefense vaccines were designed by using either live attenuated or inactivated vaccine approaches. As discussed in the above sections, live attenuated vaccines have the benefit of normally inducing stronger protective immunity than inactivated vaccines but are not ideal candidates when facing the challenges related to manufacturing processes or more critically, to improve the overall safety profile of biodefense vaccines. Furthermore, regulatory authorities expect to see the mechanism of attenuation to be well-characterized. On the other hand, inactivated vaccines, although safe in general, are usually not very immunogenic, require multiple immunizations to reach the protective levels of immunity, and have not been shown to be good inducers of T cell immunity when used alone.

Given that it takes 18-20 years to develop a vaccine in many cases, there are major initiatives to develop new platform technologies where selected protective antigens, or genes for such antigens, but not the whole pathogen as in the cases of live attenuated and inactivated vaccines, can be incorporated to generate a vaccine against a biodefense agent at short notice. At the present time, there is still a long way to achieve this goal but there are a number of promising platform technologies.

In the near term, recombinant protein-based vaccines are attractive alternatives because they can be produced using a highly standardized manufacturing process, are safer than using the entire pathogen (either live or inactivated), and should be no less immunogenic than inactivated vaccines if a proper adjuvant is included in the final formulation. Examples of the successes of such platform technology in the public health arena are hepatitis B virus (HBV) and human papillomavirus (HPV) vaccines. Recombinant protein-based vaccines represent several forerunners of the newer generation of biodefense vaccines, especially for bacterial pathogens with well-characterized protective antigens. For plague vaccines, recombinant proteins F1 and V have been well-established as key protective antigens and vaccines based on these proteins have entered clinical studies [16]. Vaccines based on the recombinant protein PA antigen have been the leading candidate for a newer generation of anthrax vaccines [8]. For certain toxin-producing bacterial pathogens, recombinant protein-based subunit vaccines based on modified toxins are also replacing the traditional toxoid as the protective antigen, such as in the case of *Botulinum* vaccines [17]. Despite the advantages for recombinant protein vaccines, one key weakness for subunit-based vaccines is their poor immunogenicity for T cell responses.

Gene-based vaccines, on the other hand, have emerged in the last decade as a completely novel strategy for vaccination [18, 19]. At first, their ability to induce antigen-specific T cell responses was considered as the main strength. Over time, however, it became clear that gene-based vaccines are also effective in eliciting antibody responses. Gene-based vaccines include DNA vaccines and vector-based vaccines. Vectors can be either viral or bacterial, but more biodefense vaccines use viral vectors over bacterial vectors. Although both DNA vaccines and vector-based vaccines incorporate a natural or modified gene from a pathogen, which encodes the protective antigen, they differ in many ways. First, DNA vaccines can be delivered directly in the form of plasmids whereas the vector approach usually required the production of a large stock of highly concentrated, packaged vector vaccines. Second, most DNA vaccines generally do not contain unrelated proteins in the construct. The vaccinated

hosts will only generate immune responses against the biodefense antigen expressed by the DNA vaccines. In contrast, vector-based vaccines express other antigens as part of the original vector virus or bacteria. Immune responses against vector components can generate several negative effects. For hosts who have been exposed to the same vector in the past, such as in the case serotype 5 of adenovirus (Ad5), pre-existing immune responses against the Ad5 can reduce the ability of a vaccine using Ad5 vector as the delivery system [20]. Anti-vector immunity may also interfere with protection of vector-based vaccines, as was observed during the STEP trial, a large international HIV vaccine clinical trial co-sponsored by the National Institute of Allergy and Infectious Diseases and the pharmaceutical company Merck & Co. Inc., in which people with high pre-existing anti-Ad5 antibody responses had a higher chance of being infected by HIV-1 when these people were immunized with an Ad5-based HIV-1 vaccine [21].

As shown in Table 2, the DNA vaccine approach has been tested for almost every biodefense pathogen due to the relatively simple nature of this approach. Vector-based vaccines have also been tested for many biodefense pathogens. We have included in Table 2 key references of published work on these gene-based vaccination approaches for each of the key pathogens included in the current Supplement. Most of these studies were conducted in small animal models and were successful in eliciting positive immunogenicity results against these biodefense pathogens, and some obtained data on protection in the cases where validated animal models exist.

The key information missing for many of these studies is the comparison between gene-based vaccines and other vaccines using traditional approaches. Such comparison would provide valuable information on the actual improvement of immunogenicity with these gene-based vaccination approaches over the existing and imperfect vaccines. However, the main challenge for gene-based vaccines is the overall low immunogenicity demonstrated in human studies for these vaccines [22]. But significant progress has been made in at least three key areas in recent years to address this issue. Molecular adjuvants, in the form of genes coding for immune-stimulating cytokines, have been shown to significantly improve the overall immunogenicity of DNA vaccines in non-human primate models [23, 24]. Physical delivery approaches, such as gene gun and electroporation, were shown more effective in eliciting higher levels of immune responses than the traditional needle injection method [25, 26]. Finally, the prime-boost strategies in which DNA or vector based vaccines were used in combination with another form of vaccines, such as in the case of DNA prime-protein boost [27], or DNA prime-Ad5 vector boost [28], have shown real promise in eliciting high and balanced antibody and T cell immune responses in both animal and human studies.

Future outlook

Since 2001, biodefense vaccine development has experienced significant progress in the areas of manufacturing, immunological mechanisms and novel vaccination approaches. There are other unique issues not covered in this technology review but they are equally important for biodefense vaccine development. These include 1) “animal rule” which applies to vaccines unable to conduct a late phase large scale efficacy trial in humans due to the rare natural occurrence of such infections; 2) bio-engineered threats which constitute a unique bioterrorism threat not belonging to the traditional pathogens; and 3) rules and process governing the use of IND (investigational new drug) status vaccines which can be developed and stockpiled for Emergency Use Authorization (EUA) purpose as established by the Project BioShield Act of 2004 (Public Law 108-276). Future successful biodefense vaccines need to be innovative to satisfy increasingly demanding requirements in safety and

efficacy. It is less likely that one format will fit every biodefense vaccine, but the future successful biodefense vaccines no doubt will incorporate many technological innovations.

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References

1. Henderson, DA.; Borio, LL.; Grabenstein, JD. Smallpox and vaccinia. In: Plotkin, SA.; Orenstein, WA.; Offit, PA., editors. *Vaccines*. Fifth. Elsevier; 2008. p. 773-804.
2. Mora LF, Khan AH, Sperling LS. Cardiac Complications after Smallpox Vaccination. *Southern medical journal*. 2009 May 7.
3. Cassimatis DC, Atwood JE, Engler RM, Linz PE, Grabenstein JD, Vernalis MN. Smallpox vaccination and myopericarditis: a clinical review. *Journal of the American College of Cardiology*. 2004 May 5; 43(9):1503–10. [PubMed: 15120802]
4. Kennedy JS, Co M, Green S, Longtine K, Longtine J, O'Neill MA, et al. The safety and tolerability of an HIV-1 DNA prime-protein boost vaccine (DP6-001) in healthy adult volunteers. *Vaccine*. 2008 Aug 18; 26(35):4420–4. [PubMed: 18588934]
5. Withdrawal of rotavirus vaccine recommendation. *Mmwr*. 1999 Nov 5.48(43):1007. [PubMed: 10577495]
6. Kingsbury N. Anthrax vaccine: changes to manufacturing process. US GAO Testimony. 2001 GAO-02-181T.
7. Greenberg RN, Kennedy JS. ACAM2000: a newly licensed cell culture-based live vaccinia smallpox vaccine. Expert opinion on investigational drugs. 2008 Apr; 17(4):555–64. [PubMed: 18363519]
8. Gorse GJ, Keitel W, Keyserling H, Taylor DN, Lock M, Alves K, et al. Immunogenicity and tolerance of ascending doses of a recombinant protective antigen (rPA102) anthrax vaccine: a randomized, double-blinded, controlled, multicenter trial. *Vaccine*. 2006 Aug 14; 24(33-34):5950–9. [PubMed: 16797805]
9. Lu D, Hickey AJ. Pulmonary vaccine delivery. Expert review of vaccines. 2007 Apr; 6(2):213–26. [PubMed: 17408371]
10. Uddowla S, Freytag LC, Clements JD. Effect of adjuvants and route of immunizations on the immune response to recombinant plague antigens. *Vaccine*. 2007 Nov 19; 25(47):7984–93. [PubMed: 17933440]
11. Hoffmann, E.; Neumann, G.; Kawaoka, Y.; Hobom, G.; Webster, RG. A DNA transfection system for generation of influenza A virus from eight plasmids; *Proceedings of the National Academy of Sciences of the United States of America*; 2000 May 23; p. 6108-13.
12. Neumann, G.; Watanabe, T.; Ito, H.; Watanabe, S.; Goto, H.; Gao, P., et al. Generation of influenza A viruses entirely from cloned cDNAs; *Proceedings of the National Academy of Sciences of the United States of America*; 1999 Aug 3; p. 9345-50.
13. Parent MA, Berggren KN, Kummer LW, Wilhelm LB, Szaba FM, Mullarky IK, et al. Cell-mediated protection against pulmonary *Yersinia pestis* infection. *Infection and immunity*. 2005 Nov; 73(11):7304–10. [PubMed: 16239527]
14. Belyakov, IM.; Earl, P.; Dzutsev, A.; Kuznetsov, VA.; Lemon, M.; Wyatt, LS., et al. Shared modes of protection against poxvirus infection by attenuated and conventional smallpox vaccine viruses; *Proceedings of the National Academy of Sciences of the United States of America*; 2003 Aug 5; p. 9458-63.
15. Demkowicz WE Jr, Littau RA, Wang J, Ennis FA. Human cytotoxic T-cell memory: long-lived responses to vaccinia virus. *Journal of virology*. 1996 Apr; 70(4):2627–31. [PubMed: 8642697]
16. Williamson ED, Flick-Smith HC, Lebutt C, Rowland CA, Jones SM, Waters EL, et al. Human immune response to a plague vaccine comprising recombinant F1 and V antigens. *Infection and immunity*. 2005 Jun; 73(6):3598–608. [PubMed: 15908389]

17. Smith LA, Rusnak JM. Botulinum neurotoxin vaccines: past, present, and future. *Critical reviews in immunology*. 2007; 27(4):303–18. [PubMed: 18197811]
18. Robinson HL. Nucleic acid vaccines: an overview. *Vaccine*. 1997 Jun; 15(8):785–7. [PubMed: 9234513]
19. Polo JM, Dubensky TW Jr. Virus-based vectors for human vaccine applications. *Drug discovery today*. 2002 Jul 1; 7(13):719–27. [PubMed: 12110228]
20. Papp Z, Babiuk LA, Baca-Estrada ME. The effect of pre-existing adenovirus-specific immunity on immune responses induced by recombinant adenovirus expressing glycoprotein D of bovine herpesvirus type 1. *Vaccine*. 1999 Feb 26; 17(7-8):933–43. [PubMed: 10067700]
21. Cohen, J. *Science*. Vol. 318. New York, NY; 2007 Nov 16. AIDS research. Did Merck's failed HIV vaccine cause harm?; p. 1048-9.
22. Lu S, Wang S, Grimes-Serrano JM. Current progress of DNA vaccine studies in humans. *Expert review of vaccines*. 2008 Mar; 7(2):175–91. [PubMed: 18324888]
23. Chong SY, Egan MA, Kutzler MA, Megati S, Masood A, Roopchand V, et al. Comparative ability of plasmid IL-12 and IL-15 to enhance cellular and humoral immune responses elicited by a SIVgag plasmid DNA vaccine and alter disease progression following SHIV(89.6P) challenge in rhesus macaques. *Vaccine*. 2007 Jun 21; 25(26):4967–82. [PubMed: 17335943]
24. Boyer, JD.; Robinson, TM.; Kutzler, MA.; Vansant, G.; Hokey, DA.; Kumar, S., et al. Protection against simian/human immunodeficiency virus (SHIV) 89.6P in macaques after coimmunization with SHIV antigen and IL-15 plasmid; *Proceedings of the National Academy of Sciences of the United States of America*; 2007 Nov 20; p. 18648-53.
25. Wang S, Zhang C, Zhang L, Li J, Huang Z, Lu S. The relative immunogenicity of DNA vaccines delivered by the intramuscular needle injection, electroporation and gene gun methods. *Vaccine*. 2008 Apr 16; 26(17):2100–10. [PubMed: 18378365]
26. Jones S, Evans K, McElwaine-Johnn H, Sharpe M, Oxford J, Lambkin-Williams R, et al. DNA vaccination protects against an influenza challenge in a double-blind randomised placebo-controlled phase 1b clinical trial. *Vaccine*. 2009 Apr 21; 27(18):2506–12. [PubMed: 19368793]
27. Wang S, Kennedy JS, West K, Montefiori DC, Coley S, Lawrence J, et al. Cross-subtype antibody and cellular immune responses induced by a polyvalent DNA prime-protein boost HIV-1 vaccine in healthy human volunteers. *Vaccine*. 2008 Jul 23; 26(31):3947–57. [PubMed: 18724414]
28. Barouch DH, McKay PF, Sumida SM, Santra S, Jackson SS, Gorgone DA, et al. Plasmid chemokines and colony-stimulating factors enhance the immunogenicity of DNA priming-viral vector boosting human immunodeficiency virus type 1 vaccines. *Journal of virology*. 2003 Aug; 77(16):8729–35. [PubMed: 12885892]
29. Grabenstein JD. Vaccines: countering anthrax: vaccines and immunoglobulins. *Clin Infect Dis*. 2008 Jan 1; 46(1):129–36. [PubMed: 18171228]
30. Brey RN. Molecular basis for improved anthrax vaccines. *Advanced drug delivery reviews*. 2005 Jun 17; 57(9):1266–92. [PubMed: 15935874]
31. Herrmann JE, Wang S, Zhang C, Panchal RG, Bavari S, Lyons CR, et al. Passive immunotherapy of *Bacillus anthracis* pulmonary infection in mice with antisera produced by DNA immunization. *Vaccine*. 2006 Jul 26; 24(31-32):5872–80. [PubMed: 16790303]
32. Little SF. Anthrax vaccines: a development update. *BioDrugs*. 2005; 19(4):233–45. [PubMed: 16128606]
33. Skoble J, Beaber JW, Gao Y, Lovchik JA, Sower LE, Liu W, et al. Killed but metabolically active *Bacillus anthracis* vaccines induce broad and protective immunity against anthrax. *Infection and immunity*. 2009 Apr; 77(4):1649–63. [PubMed: 19168734]
34. Osorio M, Wu Y, Singh S, Merkel TJ, Bhattacharyya S, Blake MS, et al. Anthrax protective antigen delivered by *Salmonella enterica* serovar Typhi Ty21a protects mice from a lethal anthrax spore challenge. *Infection and immunity*. 2009 Apr; 77(4):1475–82. [PubMed: 19179420]
35. Gauthier YP, Tournier JN, Paucod JC, Corre JP, Mock M, Goossens PL, et al. Efficacy of a vaccine based on protective antigen and killed spores against experimental inhalational anthrax. *Infection and immunity*. 2009 Mar; 77(3):1197–207. [PubMed: 19114543]

36. Trollet C, Pereira Y, Burgain A, Litzler E, Mezrahi M, Seguin J, et al. Generation of high-titer neutralizing antibodies against botulinum toxins A, B, and E by DNA electrotransfer. *Infection and immunity*. 2009 May; 77(5):2221–9. [PubMed: 19237523]
37. Yu YZ, Zhang SM, Sun ZW, Wang S, Yu WY. Enhanced immune responses using plasmid DNA replicon vaccine encoding the Hc domain of *Clostridium botulinum* neurotoxin serotype A. *Vaccine*. 2007 Dec 17; 25(52):8843–50. [PubMed: 18022294]
38. Baldwin MR, Tepp WH, Przedpelski A, Pier CL, Bradshaw M, Johnson EA, et al. Subunit vaccine against the seven serotypes of botulism. *Infection and immunity*. 2008 Mar; 76(3):1314–8. [PubMed: 18070903]
39. Jones RG, Liu Y, Rigsby P, Sesardic D. An improved method for development of toxoid vaccines and antitoxins. *Journal of immunological methods*. 2008 Aug 20; 337(1):42–8. [PubMed: 18571196]
40. Xu Q, Pichichero ME, Simpson LL, Elias M, Smith LA, Zeng M. An adenoviral vector-based mucosal vaccine is effective in protection against botulism. *Gene therapy*. 2009 Mar; 16(3):367–75. [PubMed: 19129860]
41. Zeng M, Xu Q, Elias M, Pichichero ME, Simpson LL, Smith LA. Protective immunity against botulism provided by a single dose vaccination with an adenovirus-vectored vaccine. *Vaccine*. 2007 Oct 23; 25(43):7540–8. [PubMed: 17897756]
42. Titball RW, Williamson ED. Vaccination against bubonic and pneumonic plague. *Vaccine*. 2001 Jul 20; 19(30):4175–84. [PubMed: 11457543]
43. Titball RW, Williamson ED. *Yersinia pestis* (plague) vaccines. Expert opinion on biological therapy. 2004 Jun; 4(6):965–73. [PubMed: 15174978]
44. Chichester JA, Musiychuk K, Farrance CE, Mett V, Lyons J, Mett V, et al. A single component two-valent LcrV-F1 vaccine protects non-human primates against pneumonic plague. *Vaccine*. 2009 May 26; 27(25-26):3471–4. [PubMed: 19200825]
45. Leary SE, Griffin KF, Garmory HS, Williamson ED, Titball RW. Expression of an F1/V fusion protein in attenuated *Salmonella typhimurium* and protection of mice against plague. *Microbial pathogenesis*. 1997 Sep; 23(3):167–79. [PubMed: 9281474]
46. Wang S, Heilman D, Liu F, Giehl T, Joshi S, Huang X, et al. A DNA vaccine producing LcrV antigen in oligomers is effective in protecting mice from lethal mucosal challenge of plague. *Vaccine*. 2004 Sep 3; 22(25-26):3348–57. [PubMed: 15308359]
47. Wang S, Joshi S, Mboudjeka I, Liu F, Ling T, Goguen JD, et al. Relative immunogenicity and protection potential of candidate *Yersinia Pestis* antigens against lethal mucosal plague challenge in Balb/C mice. *Vaccine*. 2008 Mar 20; 26(13):1664–74. [PubMed: 18291562]
48. Conlan JW. Vaccines against *Francisella tularensis*--past, present and future. Expert review of vaccines. 2004 Jun; 3(3):307–14. [PubMed: 15176947]
49. Bitsaktsis C, Rawool DB, Li Y, Kurkure NV, Iglesias B, Gosselin EJ. Differential requirements for protection against mucosal challenge with *Francisella tularensis* in the presence versus absence of cholera toxin B and inactivated *F. tularensis*. *J Immunol*. 2009 Apr 15; 182(8):4899–909. [PubMed: 19342669]
50. Lavine CL, Clinton SR, Angelova-Fischer I, Marion TN, Bina XR, Bina JE, et al. Immunization with heat-killed *Francisella tularensis* LVS elicits protective antibody-mediated immunity. *European journal of immunology*. 2007 Nov; 37(11):3007–20. [PubMed: 17960662]
51. Fortier AH, Slayter MV, Ziemba R, Meltzer MS, Nacy CA. Live vaccine strain of *Francisella tularensis*: infection and immunity in mice. *Infection and immunity*. 1991 Sep; 59(9):2922–8. [PubMed: 1879918]
52. El Sahly HM, Atmar RL, Patel SM, Wells JM, Cate T, Ho M, et al. Safety, reactogenicity and immunogenicity of *Francisella tularensis* live vaccine strain in humans. *Vaccine*. 2009 Aug 6; 27(36):4905–11. [PubMed: 19567246]
53. Gregory SH, Mott S, Phung J, Lee J, Moise L, McMurry JA, et al. Epitope-based vaccination against pneumonic tularemia. *Vaccine*. 2009 Jul 16.
54. Sjostedt A, Sandstrom G, Tarnvik A. Humoral and cell-mediated immunity in mice to a 17-kilodalton lipoprotein of *Francisella tularensis* expressed by *Salmonella typhimurium*. *Infection and immunity*. 1992 Jul; 60(7):2855–62. [PubMed: 1612751]

55. Dreisbach VC, Cowley S, Elkins KL. Purified lipopolysaccharide from *Francisella tularensis* live vaccine strain (LVS) induces protective immunity against LVS infection that requires B cells and gamma interferon. *Infection and immunity*. 2000 Apr; 68(4):1988–96. [PubMed: 10722593]
56. Huntley JF, Conley PG, Rasko DA, Hagman KE, Apicella MA, Norgard MV. Native outer membrane proteins protect mice against pulmonary challenge with virulent type A *Francisella tularensis*. *Infection and immunity*. 2008 Aug; 76(8):3664–71. [PubMed: 18505805]
57. Hoover DL, Crawford RM, Van De Verg LL, Izadjoo MJ, Bhattacharjee AK, Paravitana CM, et al. Protection of mice against brucellosis by vaccination with *Brucella melitensis* WR201(16MDeltapurEK). *Infection and immunity*. 1999 Nov; 67(11):5877–84. [PubMed: 10531243]
58. Estevan M, Gamazo C, Grillo MJ, Del Barrio GG, Blasco JM, Irache JM. Experiments on a sub-unit vaccine encapsulated in microparticles and its efficacy against *Brucella melitensis* in mice. *Vaccine*. 2006 May 8; 24(19):4179–87. [PubMed: 16481077]
59. Vemulapalli R, Contreras A, Sanakkayala N, Sriranganathan N, Boyle SM, Schurig GG. Enhanced efficacy of recombinant *Brucella abortus* RB51 vaccines against *B. melitensis* infection in mice. *Veterinary microbiology*. 2004 Sep 8; 102(3-4):237–45. [PubMed: 15327798]
60. Dieterich RA, Deyoe BL, Morton JK. Effects of killed *Brucella abortus* strain 45/20 vaccine on reindeer later challenge exposed with *Brucella suis* type 4. *American journal of veterinary research*. 1981 Jan; 42(1):131–4. [PubMed: 6784617]
61. Arenas-Gamboa AM, Ficht TA, Kahl-McDonagh MM, Rice-Ficht AC. Immunization with a single dose of a microencapsulated *Brucella melitensis* mutant enhances protection against wild-type challenge. *Infection and immunity*. 2008 Jun; 76(6):2448–55. [PubMed: 18362129]
62. Yu DH, Hu XD, Cai H. A combined DNA vaccine encoding BCSP31, SOD, and L7/L12 confers high protection against *Brucella abortus* 2308 by inducing specific CTL responses. *DNA and cell biology*. 2007 Jun; 26(6):435–43. [PubMed: 17570767]
63. Luo D, Ni B, Li P, Shi W, Zhang S, Han Y, et al. Protective immunity elicited by a divalent DNA vaccine encoding both the L7/L12 and Omp16 genes of *Brucella abortus* in BALB/c mice. *Infection and immunity*. 2006 May; 74(5):2734–41. [PubMed: 16622210]
64. Cassataro J, Estein SM, Pasquevich KA, Velikovskiy CA, de la Barrera S, Bowden R, et al. Vaccination with the recombinant *Brucella* outer membrane protein 31 or a derived 27-amino-acid synthetic peptide elicits a CD4+ T helper 1 response that protects against *Brucella melitensis* infection. *Infection and immunity*. 2005 Dec; 73(12):8079–88. [PubMed: 16299302]
65. Harms JS, Durward MA, Magnani DM, Splitter GA. Evaluation of recombinant invasive, non-pathogenic *Escherichia coli* as a vaccine vector against the intracellular pathogen, *Brucella*. *Journal of immune based therapies and vaccines*. 2009; 7:1. [PubMed: 19126207]
66. Neeson BN, Clark GC, Atkins HS, Lingard B, Titball RW. Analysis of protection afforded by a *Clostridium perfringens* alpha-toxoid against heterologous clostridial phospholipases C. *Microbial pathogenesis*. 2007 Oct; 43(4):161–5. [PubMed: 17604945]
67. Williamson ED, Titball RW. A genetically engineered vaccine against the alpha-toxin of *Clostridium perfringens* protects mice against experimental gas gangrene. *Vaccine*. 1993 Sep; 11(12):1253–8. [PubMed: 8256506]
68. Stevens DL, Titball RW, Jepson M, Bayer CR, Hayes-Schroer SM, Bryant AE. Immunization with the C-Domain of alpha -Toxin prevents lethal infection, localizes tissue injury, and promotes host response to challenge with *Clostridium perfringens*. *The Journal of infectious diseases*. 2004 Aug 15; 190(4):767–73. [PubMed: 15272405]
69. Zekarias B, Mo H, Curtiss R 3rd. Recombinant attenuated *Salmonella enterica* serovar typhimurium expressing the carboxy-terminal domain of alpha toxin from *Clostridium perfringens* induces protective responses against necrotic enteritis in chickens. *Clin Vaccine Immunol*. 2008 May; 15(5):805–16. [PubMed: 18337376]
70. Richards AL. Rickettsial vaccines: the old and the new. *Expert review of vaccines*. 2004 Oct; 3(5): 541–55. [PubMed: 15485334]
71. Sumner JW, Sims KG, Jones DC, Anderson BE. Protection of guinea-pigs from experimental Rocky Mountain spotted fever by immunization with baculovirus-expressed *Rickettsia rickettsii* rOmpA protein. *Vaccine*. 1995 Jan; 13(1):29–35. [PubMed: 7762273]

72. Clements ML, Wisseman CL Jr, Woodward TE, Fiset P, Dumler JS, McNamee W, et al. Reactogenicity, immunogenicity, and efficacy of a chick embryo cell-derived vaccine for Rocky Mountain spotted fever. *The Journal of infectious diseases*. 1983 Nov; 148(5):922–30. [PubMed: 6415182]
73. Dumler JS, Wisseman CL Jr, Fiset P, Clements ML. Cell-mediated immune responses of adults to vaccination, challenge with *Rickettsia rickettsii*, or both. *The American journal of tropical medicine and hygiene*. 1992 Feb; 46(2):105–15. [PubMed: 1539743]
74. Coker C, Majid M, Radulovic S. Development of *Rickettsia prowazekii* DNA vaccine: cloning strategies. *Annals of the New York Academy of Sciences*. 2003 Jun; 990:757–64. [PubMed: 12860719]
75. Wisseman CL Jr. Concepts of louse-borne typhus control in developing countries: the use of the living attenuated E strain typhus vaccine in epidemic and endemic situations. *Advances in experimental medicine and biology*. 1972; 31(0):97–130. [PubMed: 4211883]
76. Mason RA, Wenzel RP, Seligmann EB Jr, Ginn RK. A reference, inactivated, epidemic typhus vaccine: clinical trials in man. *Journal of biological standardization*. 1976; 4(3):217–24. [PubMed: 820697]
77. Greenberg RN, Kennedy JS, Clanton DJ, Plummer EA, Hague L, Cruz J, et al. Safety and immunogenicity of new cell-cultured smallpox vaccine compared with calf-lymph derived vaccine: a blind, single-centre, randomised controlled trial. *Lancet*. 2005; 365(9457):398–409. [PubMed: 15680454]
78. Ravanfar P, Satyaprakash A, Creed R, Mendoza N. Existing antiviral vaccines. *Dermatologic therapy*. 2009 Mar-Apr; 22(2):110–28. [PubMed: 19335723]
79. Kennedy RB, Ovsyannikova IG, Jacobson RM, Poland GA. The immunology of smallpox vaccines. *Current opinion in immunology*. 2009 Jun; 21(3):314–20. [PubMed: 19524427]
80. Sakhatskyy P, Wang S, Zhang C, Chou TH, Kishko M, Lu S. Immunogenicity and protection efficacy of subunit-based smallpox vaccines using variola major antigens. *Virology*. 2008 Feb 5; 371(1):98–107. [PubMed: 17950773]
81. Sakhatskyy P, Wang S, Chou TH, Lu S. Immunogenicity and protection efficacy of monovalent and polyvalent poxvirus vaccines that include the D8 antigen. *Virology*. 2006 Nov 25; 355(2):164–74. [PubMed: 16919703]
82. Jacobs BL, Langland JO, Kibler KV, Denzler KL, White SD, Holechek SA, et al. Vaccinia virus vaccines: Past, present and future. *Antiviral research*. 2009 Jun 26.
83. Artenstein AW. New generation smallpox vaccines: a review of preclinical and clinical data. *Reviews in medical virology*. 2008 Jul-Aug; 18(4):217–31. [PubMed: 18283712]
84. Bielinska AU, Chepurinov AA, Landers JJ, Janczak KW, Chepurnova TS, Luker GD, et al. A novel, killed-virus nasal vaccinia virus vaccine. *Clin Vaccine Immunol*. 2008 Feb; 15(2):348–58. [PubMed: 18057181]
85. Cho HW, Howard CR, Lee HW. Review of an inactivated vaccine against hantaviruses. *Intervirology*. 2002; 45(4-6):328–33. [PubMed: 12602351]
86. Hjelle B. Vaccines against hantaviruses. *Expert review of vaccines*. 2002 Oct; 1(3):373–84. [PubMed: 12901576]
87. Yamanishi K, Tanishita O, Tamura M, Asada H, Kondo K, Takagi M, et al. Development of inactivated vaccine against virus causing haemorrhagic fever with renal syndrome. *Vaccine*. 1988 Jun; 6(3):278–82. [PubMed: 3138829]
88. Maes P, Clement J, Van Ranst M. Recent approaches in hantavirus vaccine development. *Expert review of vaccines*. 2009 Jan; 8(1):67–76. [PubMed: 19093774]
89. Hooper JW, Custer DM, Smith J, Wahl-Jensen V. Hantaan/Andes virus DNA vaccine elicits a broadly cross-reactive neutralizing antibody response in nonhuman primates. *Virology*. 2006 Mar 30; 347(1):208–16. [PubMed: 16378630]
90. Schmaljohn CS, Hasty SE, Dalrymple JM. Preparation of candidate vaccinia-vectored vaccines for haemorrhagic fever with renal syndrome. *Vaccine*. 1992; 10(1):10–3. [PubMed: 1347189]
91. Sohn YM, Rho HO, Park MS, Kim JS, Summers PL. Primary humoral immune responses to formalin inactivated hemorrhagic fever with renal syndrome vaccine (Hantavax): consideration of

- active immunization in South Korea. *Yonsei medical journal*. 2001 Jun; 42(3):278–84. [PubMed: 11456392]
92. Maes P, Keyaerts E, Bonnet V, Clement J, Avsic-Zupanc T, Robert A, et al. Truncated recombinant Dobrava hantavirus nucleocapsid proteins induce strong, long-lasting immune responses in mice. *Intervirology*. 2006; 49(5):253–60. [PubMed: 16714853]
 93. Sullivan NJ, Sanchez A, Rollin PE, Yang ZY, Nabel GJ. Development of a preventive vaccine for Ebola virus infection in primates. *Nature*. 2000 Nov 30; 408(6812):605–9. [PubMed: 11117750]
 94. Yang ZY, Wyatt LS, Kong WP, Moodie Z, Moss B, Nabel GJ. Overcoming immunity to a viral vaccine by DNA priming before vector boosting. *Journal of virology*. 2003 Jan; 77(1):799–803. [PubMed: 12477888]
 95. Bukreyev A, Marzi A, Feldmann F, Zhang L, Yang L, Ward JM, et al. Chimeric human parainfluenza virus bearing the Ebola virus glycoprotein as the sole surface protein is immunogenic and highly protective against Ebola virus challenge. *Virology*. 2009 Jan 20; 383(2):348–61. [PubMed: 19010509]
 96. Warfield KL, Swenson DL, Olinger GG, Kalina WV, Aman MJ, Bavari S. Ebola virus-like particle-based vaccine protects nonhuman primates against lethal Ebola virus challenge. *The Journal of infectious diseases*. 2007 Nov 15; 196(2):S430–7. [PubMed: 17940980]
 97. Yang C, Ye L, Compans RW. Protection against filovirus infection: virus-like particle vaccines. Expert review of vaccines. 2008 Apr; 7(3):333–44. [PubMed: 18393603]
 98. Hevey M, Negley D, Geisbert J, Jahrling P, Schmaljohn A. Antigenicity and vaccine potential of Marburg virus glycoprotein expressed by baculovirus recombinants. *Virology*. 1997 Dec 8; 239(1):206–16. [PubMed: 9426460]
 99. Hevey M, Negley D, VanderZanden L, Tammariello RF, Geisbert J, Schmaljohn C, et al. Marburg virus vaccines: comparing classical and new approaches. *Vaccine*. 2001 Nov 12; 20(3-4):586–93. [PubMed: 11672925]
 100. Sheets RL, Stein J, Bailer RT, Koup RA, Andrews C, Nason M, et al. Biodistribution and toxicological safety of adenovirus type 5 and type 35 vectored vaccines against human immunodeficiency virus-1 (HIV-1), Ebola, or Marburg are similar despite differing adenovirus serotype vector, manufacturer's construct, or gene inserts. *Journal of immunotoxicology*. 2008 Jul; 5(3):315–35. [PubMed: 18830892]
 101. Daddario-DiCaprio KM, Geisbert TW, Stroher U, Geisbert JB, Grolla A, Fritz EA, et al. Postexposure protection against Marburg haemorrhagic fever with recombinant vesicular stomatitis virus vectors in non-human primates: an efficacy assessment. *Lancet*. 2006 Apr 29; 367(9520):1399–404. [PubMed: 16650649]
 102. Pittman PR, Liu CT, Cannon TL, Makuch RS, Mangiafico JA, Gibbs PH, et al. Immunogenicity of an inactivated Rift Valley fever vaccine in humans: a 12-year experience. *Vaccine*. 1999 Aug 20; 18(1-2):181–9. [PubMed: 10501248]
 103. The use of veterinary vaccines for prevention and control of Rift Valley fever: memorandum from a WHO/FAO meeting. *Bulletin of the World Health Organization*. 1983; 61(2):261–8. [PubMed: 6602663]
 104. Caplen H, Peters CJ, Bishop DH. Mutagen-directed attenuation of Rift Valley fever virus as a method for vaccine development. *The Journal of general virology*. 1985 Oct; 66(Pt 10):2271–7. [PubMed: 4045430]
 105. Schmaljohn CS, Parker MD, Ennis WH, Dalrymple JM, Collett MS, Suzich JA, et al. Baculovirus expression of the M genome segment of Rift Valley fever virus and examination of antigenic and immunogenic properties of the expressed proteins. *Virology*. 1989 May; 170(1):184–92. [PubMed: 2655274]
 106. Gorchakov R, Volkova E, Yun N, Petrakova O, Linde NS, Paessler S, et al. Comparative analysis of the alphavirus-based vectors expressing Rift Valley fever virus glycoproteins. *Virology*. 2007 Sep 15; 366(1):212–25. [PubMed: 17507072]
 107. Lagerqvist N, Naslund J, Lundkvist A, Bouloy M, Ahlm C, Bucht G. Characterisation of immune responses and protective efficacy in mice after immunisation with Rift Valley Fever virus cDNA constructs. *Virology journal*. 2009; 6:6. [PubMed: 19149901]

108. Naslund J, Lagerqvist N, Habjan M, Lundkvist A, Evander M, Ahlm C, et al. Vaccination with virus-like particles protects mice from lethal infection of Rift Valley Fever Virus. *Virology*. 2009 Mar 15; 385(2):409–15. [PubMed: 19157482]
109. Fine DL, Roberts BA, Teehee ML, Terpening SJ, Kelly CL, Raetz JL, et al. Venezuelan equine encephalitis virus vaccine candidate (V3526) safety, immunogenicity and efficacy in horses. *Vaccine*. 2007 Feb 26; 25(10):1868–76. [PubMed: 17240002]
110. Dupuy LC, Locher CP, Paidhungat M, Richards MJ, Lind CM, Bakken R, et al. Directed molecular evolution improves the immunogenicity and protective efficacy of a Venezuelan equine encephalitis virus DNA vaccine. *Vaccine*. 2009 Jun 24; 27(31):4152–60. [PubMed: 19406186]
111. Pittman PR, Makuch RS, Mangiafico JA, Cannon TL, Gibbs PH, Peters CJ. Long-term duration of detectable neutralizing antibodies after administration of live-attenuated VEE vaccine and following booster vaccination with inactivated VEE vaccine. *Vaccine*. 1996 Mar; 14(4):337–43. [PubMed: 8744562]
112. Edelman R, Ascher MS, Oster CN, Ramsburg HH, Cole FE, Eddy GA. Evaluation in humans of a new, inactivated vaccine for Venezuelan equine encephalitis virus (C-84). *The Journal of infectious diseases*. 1979 Nov; 140(5):708–15. [PubMed: 528788]
113. Bennett AM, Phillpotts RJ, Perkins SD, Jacobs SC, Williamson ED. Gene gun mediated vaccination is superior to manual delivery for immunisation with DNA vaccines expressing protective antigens from *Yersinia pestis* or Venezuelan Equine Encephalitis virus. *Vaccine*. 1999 Nov 12; 18(7-8):588–96. [PubMed: 10547416]
114. Hodgson LA, Ludwig GV, Smith JF. Expression, processing, and immunogenicity of the structural proteins of Venezuelan equine encephalitis virus from recombinant baculovirus vectors. *Vaccine*. 1999 Mar 5; 17(9-10):1151–60. [PubMed: 10195627]
115. Kinney RM, Esposito JJ, Mathews JH, Johnson BJ, Roehrig JT, Barrett AD, et al. Recombinant vaccinia virus/Venezuelan equine encephalitis (VEE) virus protects mice from peripheral VEE virus challenge. *Journal of virology*. 1988 Dec; 62(12):4697–702. [PubMed: 3184276]
116. Geeraedts F, Bungener L, Pool J, ter Veer W, Wilschut J, Huckriede A. Whole inactivated virus influenza vaccine is superior to subunit vaccine in inducing immune responses and secretion of proinflammatory cytokines by DCs. *Influenza and other respiratory viruses*. 2008 Mar; 2(2):41–51. [PubMed: 19453471]
117. Alsharifi M, Furuya Y, Bowden TR, Lobigs M, Koskinen A, Regner M, et al. Intranasal flu vaccine protective against seasonal and H5N1 avian influenza infections. *PloS one*. 2009; 4(4):e5336. [PubMed: 19401775]
118. Wang S, Parker C, Taaffe J, Solorzano A, Garcia-Sastre A, Lu S. Heterologous HA DNA vaccine prime--inactivated influenza vaccine boost is more effective than using DNA or inactivated vaccine alone in eliciting antibody responses against H1 or H3 serotype influenza viruses. *Vaccine*. 2008 Jul 4; 26(29-30):3626–33. [PubMed: 18538900]
119. Wang S, Taaffe J, Parker C, Solorzano A, Cao H, Garcia-Sastre A, et al. Hemagglutinin (HA) proteins from H1 and H3 serotypes of influenza A viruses require different antigen designs for the induction of optimal protective antibody responses as studied by codon-optimized HA DNA vaccines. *Journal of virology*. 2006 Dec; 80(23):11628–37. [PubMed: 16987975]
120. Vajo Z, Kosa L, Visontay I, Jankovics M, Jankovics I. Inactivated whole virus influenza A (H5N1) vaccine. *Emerging infectious diseases*. 2007 May; 13(5):807–8. [PubMed: 18044056]
121. Ehrlich HJ, Muller M, Oh HM, Tambyah PA, Joukhadar C, Montomoli E, et al. A clinical trial of a whole-virus H5N1 vaccine derived from cell culture. *The New England journal of medicine*. 2008 Jun 12; 358(24):2573–84. [PubMed: 18550874]
122. Treanor JJ, Schiff GM, Hayden FG, Brady RC, Hay CM, Meyer AL, et al. Safety and immunogenicity of a baculovirus-expressed hemagglutinin influenza vaccine: a randomized controlled trial. *Jama*. 2007 Apr 11; 297(14):1577–82. [PubMed: 17426277]
123. Haynes JR. Influenza virus-like particle vaccines. *Expert review of vaccines*. 2009 Apr; 8(4):435–45. [PubMed: 19348559]

124. Greenwood DL, Dynon K, Kalkanidis M, Xiang S, Plebanski M, Scheerlinck JP. Vaccination against foot-and-mouth disease virus using peptides conjugated to nano-beads. *Vaccine*. 2008 May 23; 26(22):2706–13. [PubMed: 18448209]
125. Niborski V, Li Y, Brennan F, Lane M, Torche AM, Remond M, et al. Efficacy of particle-based DNA delivery for vaccination of sheep against FMDV. *Vaccine*. 2006 Nov 30; 24(49-50):7204–13. [PubMed: 16949709]
126. Barteling SJ, Woortmeyer R. Formaldehyde inactivation of foot-and-mouth disease virus. Conditions for the preparation of safe vaccine. *Archives of virology*. 1984; 80(2-3):103–17. [PubMed: 6326708]
127. Amadori M, Barei S, Melegari M, Panina GF. Safety and efficacy of foot-and-mouth disease vaccines containing endonuclease-inactivated virions. *Vaccine*. 1987 Sep; 5(3):219–22. [PubMed: 2823495]
128. Mason PW, Piccone ME, McKenna TS, Chinsangaram J, Grubman MJ. Evaluation of a live-attenuated foot-and-mouth disease virus as a vaccine candidate. *Virology*. 1997 Jan 6; 227(1):96–102. [PubMed: 9007062]
129. Ishimaru D, Sa-Carvalho D, Silva JL. Pressure-inactivated FMDV: a potential vaccine. *Vaccine*. 2004 Jun 2; 22(17-18):2334–9. [PubMed: 15149793]
130. Zheng M, Jin N, Zhang H, Jin M, Lu H, Ma M, et al. Construction and immunogenicity of a recombinant fowlpox virus containing the capsid and 3C protease coding regions of foot-and-mouth disease virus. *Journal of virological methods*. 2006 Sep; 136(1-2):230–7. [PubMed: 16780963]
131. Du Y, Jiang P, Li Y, He H, Jiang W, Wang X, et al. Immune responses of two recombinant adenoviruses expressing VP1 antigens of FMDV fused with porcine granulocyte macrophage colony-stimulating factor. *Vaccine*. 2007 Nov 28; 25(49):8209–19. [PubMed: 17980939]
132. Li Z, Yi Y, Yin X, Zhang Z, Liu J. Expression of foot-and-mouth disease virus capsid proteins in silkworm-baculovirus expression system and its utilization as a subunit vaccine. *PloS one*. 2008; 3(5):e2273. [PubMed: 18509464]
133. Qian P, Li XM, Jin ML, Peng GQ, Chen HC. An approach to a FMD vaccine based on genetic engineered attenuated pseudorabies virus: one experiment using VP1 gene alone generates an antibody responds on FMD and pseudorabies in swine. *Vaccine*. 2004 Jun 2; 22(17-18):2129–36. [PubMed: 15149769]
134. Stott JL, Barber TL, Osburn BI. Immunologic response of sheep to inactivated and virulent bluetongue virus. *American journal of veterinary research*. 1985 May; 46(5):1043–9. [PubMed: 2988376]
135. Bhanuprakash V, Indrani BK, Hosamani M, Balamurugan V, Singh RK. Bluetongue vaccines: the past, present and future. *Expert review of vaccines*. 2009 Feb; 8(2):191–204. [PubMed: 19196199]
136. Roy P, Boyce M, Noad R. Prospects for improved bluetongue vaccines. *Nature reviews*. 2009 Feb; 7(2):120–8.
137. Campbell CH. Immunogenicity of bluetongue virus inactivated by gamma irradiation. *Vaccine*. 1985 Dec; 3(5):401–6. [PubMed: 3002061]
138. Boone JD, Balasuriya UB, Karaca K, Audonnet JC, Yao J, He L, et al. Recombinant canarypox virus vaccine co-expressing genes encoding the VP2 and VP5 outer capsid proteins of bluetongue virus induces high level protection in sheep. *Vaccine*. 2007 Jan 8; 25(4):672–8. [PubMed: 17059856]
139. Zheng YZ, Greenfield PF, Reid S. Optimized production of recombinant bluetongue core-like particles produced by the baculovirus expression system. *Biotechnology and bioengineering*. 1999 Dec 5; 65(5):600–4. [PubMed: 10516586]
140. Roy P. Genetically engineered particulate virus-like structures and their use as vaccine delivery systems. *Intervirology*. 1996; 39(1-2):62–71. [PubMed: 8957671]
141. Roy P. Multiple gene expression in baculovirus system. Third generation vaccines for bluetongue disease and African horse sickness disease. *Annals of the New York Academy of Sciences*. 1996 Jul 23; 791:318–32. [PubMed: 8784513]

142. Boyce M, Celma CC, Roy P. Development of reverse genetics systems for bluetongue virus: recovery of infectious virus from synthetic RNA transcripts. *Journal of virology*. 2008 Sep; 82(17):8339–48. [PubMed: 18562540]

Table 1
Technological issues facing the development of biodefense vaccines

	Issues
Manufacturing	Increased concern on safety of vaccines
	Production of live attenuated vaccines by cell culture approach
	Enhanced cGMP compliance
	Maintained efficacy with extended stockpiling
	Reverse genetics to produce fast and safe vaccine seed strain
	Including adjuvant as part of vaccine formulation
	Formulation of aerosol vaccines
Correlates of protection	T cell mediated vaccine protection
	Balanced antibody and T cell immunity
	Development of novel biomarker assays
Subunit or gene based vaccines	Recombinant protein vaccines: selection of expression systems and adjuvant;
	Vector based vaccine: pre-existing immunity against vector components
	Low immunity of DNA vaccine in humans by traditional delivery system.
	Prime-boost using two or more vaccine modalities.

Table 2
Candidate vaccines against key bioterrorism agents

Name of Pathogen	Classification	Previous vaccines	New vaccines under development	Ref.
Bacterial pathogens				
<i>Bacillus anthracis</i>	HHS/USDA Select Agent, CDC Category A Bioterrorism Agent	Avirulent bacterial culture extract and secreted Protective Antigen (AVA)	Subunit based vaccine; DNA vaccine; killed anthrax vaccine; killed but metabolically active <i>B. anthracis</i> vaccine; recombinant bacterial vector (Ty21a) based vaccine; killed spore vaccine	[29-35]
<i>Clostridium botulinum</i>	HHS Select Agent, CDC Category A Bioterrorism Agent	Tetavalent toxoid currently in Japan for high risk population	Toxoid; subunit-based vaccine using non-toxic toxins or fragments; DNA vaccine; viral vector based vaccine	[17, 36-41]
<i>Yersinia pestis</i>	HHS Select Agent, CDC Category A Bioterrorism Agent	Killed Whole Cell (KWC) vaccine, or live attenuated vaccine	Subunit-based vaccine; DNA vaccine; bacterial vector vaccine; live attenuated; plant-derived vaccine; viral vector based vaccine.	[16, 42-47]
<i>Francisella tularensis</i>	HHS Select Agent, CDC Category A Bioterrorism Agent	Live attenuated vaccine (LVS)	Inactivated vaccine; further attenuated live vaccine; epitope-based vaccine; bacterial vector based vaccine; subunit-based vaccine	[48-56]
<i>Brucella</i> species	HHS/USDA Select Agent, CDC Category B Bioterrorism Agent		Live attenuated vaccine; killed Brucella vaccine; subunit-based vaccine; DNA vaccine; recombinant E. coli vector vaccine	[57-65]
<i>Clostridium perfringens</i>	HHS Select Agent, CDC Category B Bioterrorism Agent	Toxoid	Subunit based non-toxic toxins or toxin fragments; recombinant Salmonella, Bacillus and vaccinia vaccine vectors.	[66-69]
Rickettsia	HHS Select Agent, CDC Category B Bioterrorism Agent	KWC and live attenuated vaccines	Live attenuated vaccine; subunit-based vaccine; DNA vaccine	[70-76]
Viral diseases				
Variola major virus	HHS Select Agent, CDC Category A Bioterrorism Agent	Live attenuated vaccines (calf lymph)	Cell culture produced live attenuated vaccines; replication defective highly attenuated vaccine; inactivated vaccines; DNA vaccine and subunit based vaccines using immunogens from Variola major virus and vaccinia; prime-boost vaccines.	[77-84]
Hantavirus	HHS Select Agent, CDC Category A Bioterrorism Agent	Inactivated hantavirus vaccine currently in Korea	Inactivated hantavirus vaccines; subunit based vaccines; viral like particles (VLP); DNA vaccines; viral vector based vaccine.	[85-92]
Ebola virus	HHS Select Agent, CDC Category A Bioterrorism Agent		Subunit-based vaccine and VLP; DNA vaccine; vector based vaccines;	[93-97]
Marburg virus	HHS Select Agent, CDC Category A Bioterrorism Agent		Inactivated vaccine; live attenuated vaccine; subunit based vaccine; viral vector based vaccine; DNA vaccine.	[98-101]
Rift Valley fever virus	HHS/USDA Overlap Select Agent,	Inactivated vaccine and live attenuated vaccines for veterinary use	Inactivated vaccine; live attenuated vaccine; subunit-based vaccine; VLP; DNA vaccine; viral vector-based vaccine.	[102-108]

Name of Pathogen	Classification	Previous vaccines	New vaccines under development	Ref.
Venezuelan equine encephalitis virus	HHS/USDA Overlap Select Agent, CDC Category B Bioterrorism Agent		Inactivated vaccines; live attenuated vaccines; DNA vaccines; viral vector vaccine; prime-boost vaccines.	[109-115]
Influenza viruses	Highly pathogenic avian influenza virus as USDA Select Agent for Livestock	Inactivated influenza vaccine; cold-adapted influenza vaccine	Inactivated vaccine; live attenuated vaccine; subunit based vaccine; VLP; DNA vaccine; viral vector based vaccine; prime-boost vaccines.	[25, 26, 116-123]
<i>Veterinary Vaccines</i>				
Foot and mouth disease virus	USDA Select Agent for Livestock	Inactivated viral vaccine	live attenuated viral vaccine; peptide conjugated vaccine; DNA vaccine; viral vector vaccines.	[124-133]
Bluetongue virus	USDA Select Agent for Livestock	Inactivated and attenuated live virus vaccines	Subunit-based vaccine; viral like particle; core-like particle; viral vector based vaccine.	[134-142]