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Advances in science and technology: Vaccine development

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I. Introduction

1. The topical scientific subject to be considered in 2013 is 'advances in technologies for surveillance, detection, diagnosis and mitigation of infectious diseases, and similar occurrences caused by toxins in humans, animals and plants'. The UK paper on scientific and technological developments submitted for the Seventh Review Conference highlighted advances in a range of technologies relevant to this topic.[1] However, this Working Paper focuses on the mitigation aspect and in particular summarises recent developments in vaccine design, production and delivery, and their implications for the BTWC.

II. Vaccine design

2. Rational vaccine design depends on an understanding of the pathogenicity of the disease agent and of the host immunological response. Rapid advances in a number of enabling technologies including the '-omics', bioinformatics, systems biology and immunology have assisted the development of new strategies, allowing the identification of new targets and reducing the timescale for vaccine development.

3. However, many challenges remain and will continue to be addressed, such as the issue of antigenic strain differences and high diversity found in some pathogens, and the

[1] BWC/CONF.VII/INF.3/Add.1, pages 21-38.

need to direct and optimise the immune response by development of adjuvants. Some examples of recent developments include:

(a) Design of self-assembling influenza nanoparticle vaccines that elicit broader and more potent immunity than traditional influenza vaccines.[2] Although not yet a universal influenza vaccine, the protein nanoparticles generated induce the production of antibodies that neutralise a broad spectrum of H1N1 viruses. In addition, the synthetic nanoparticles eliminate the need to grow potentially dangerous viruses in eggs or cell culture, a comparatively costly and time-consuming step of commercial vaccine production. This approach shows potential for a new generation of influenza vaccines and could possibly be adapted for a wide variety of pathogens. Further studies will be required to test the synthetic nanoparticles in humans and to develop cost-effective manufacturing methods.

(b) A similar approach has been used in research on HIV vaccine design, which utilised computational prediction and directed evolution to engineer an immunogen that would elicit production of broadly neutralising antibodies. A self-assembling virus-like nanoparticle was designed and coated with multiple copies of the optimised immunogen to achieve better stimulation of the antibody response. This technique in vaccine design could also be useful against other viruses with high antigenic diversity. FF[3]

(c) Demonstration of the potential of DNA nanostructures to serve as general platforms for the rational design and construction of a variety of vaccines.[4] The strategy aims to maximise vaccine immunogenicity without compromising safety by rational design of molecular complexes that mimic the natural structure of immunogenic microbes but without the disease-causing components. DNA nanostructures were used as scaffolds to assemble antigen-adjuvant complexes that elicited a strong and specific antibody response without inducing a reaction to the DNA nanostructure itself.

(d) Development of a synthetic approach that rapidly generated influenza vaccine viruses from sequence data.[5] The researchers used synthetic genomics tool to synthesize rapidly and accurately the genes encoding the two antigens, haemagglutinin (HA) and neuraminidase (NA), used in vaccine production. These genes were used to transfect a manufacturing cell line along with other improved, essential influenza viral genes, and viruses with increased yields of vaccine antigen were selected for use in vaccine development. This timed proof-of-concept study demonstrated the accurate construction of robust synthetic vaccine virus within a week. This novel method could potentially enable accelerated responses to future influenza pandemics, through instantaneous electronic sequence data exchange followed by real-time, geographically dispersed vaccine production. Following this initial study, further work is needed to prove effectiveness of manufacturing and field implementation.

(e) Creation of a synthetic vaccine for foot and mouth disease.[6] This study reconstructed the foot and mouth disease virus (FMDV) capsid from synthetic protein components, and demonstrated its ability to elicit the production of protective antibodies.

[2] Kanekiyo et al. (2013) Self-assembling influenza nanoparticle vaccines elicit broadly neutralizing H1N1 antibodies. Nature 499: 102-106 doi:10.1038/nature12202.

[3] Jardine et al. (2013) Rational HIV Immunogen Design to Target Specific Germline B Cell Receptors. Science 340:711-716 doi:10.1126/science.1234150.

[4] Liu et al. (2012) A DNA Nanostructure Platform for Directed Assembly of Synthetic Vaccines. Nano Lett. 12:4254-4259 dx.doi.org/10.1021/nl301877k.

[5] Dormitzer et al. (2013) Synthetic Generation of Influenza Vaccine Viruses for Rapid Response to Pandemics. Sci. Transl. Med. 5:185ra68 doi:10.1126/scitranslmed.3006368.

[6] Porta et al. (2013) Rational Engineering of Recombinant Picornavirus Capsids to Produce Safe, Protective Vaccine Antigen. PLoS Pathog 9(3): e1003255. doi:10.1371/journal.ppat.1003255.

Since it contains no genetic material it cannot infect animals, and would not require expensive high containment facilities for its production. In addition, steps to engineer enhanced stability characteristics into the construct mean that it would not require cold storage and that it would be cheaper to produce and distribute.

(f) Research on a surface polysaccharide common to a diverse range of microbial pathogens, including bacterial, fungal and protozoan examples, which could provide a promising target for the development of a broad spectrum vaccine.[7] A monoclonal antibody to the polysaccharide has been tested successfully in a phase I clinical trial, and a synthetic oligosaccharide conjugate vaccine is being prepared for human testing. It is yet to be determined if immunity to this polysaccharide alone would be sufficient to provide adequate protection or if it would need to be part of a multivalent vaccine. Further tests will be required for full evaluation of safety and efficacy.

III. Vaccine production

4. Traditional vaccine production methods have benefited from design improvements in fermenters and bioreactors, which have led to an increase in yield, portability and safety. Single-use or disposable bioreactor systems have also progressed; these are easily installed, reduce costs, streamline validation, increase product consistency and reduce overall turnaround times. Simple rocker bags are suited to cell-culture for virus vaccine production as an alternative to traditional methods of growth in embryonated eggs. Single-use or disposable components also feature increasingly in downstream processing equipment.

5. The widening diversity of vaccine production methods now include: cell cultures and cell suspension bioreactors; recombinant DNA; metabolic engineering and synthetic biology; chemical peptide synthesis; and transgenic animals and plants. Many developments in vaccine design result in elimination of the need for production in high containment facilities and reduction of manufacturing times. Earlier this year the FDA approved an influenza vaccine based on recombinant DNA technology and produced in insect cell lines. This technology offers the potential for faster start-up of the vaccine manufacturing process in the event of a pandemic, since it is not dependent on egg supply or live influenza virus.

6. Interest continues in the use of transgenic animals and plants for the production of therapeutic agents and vaccines. Recombinant proteins have been produced in the milk of transgenic animals, and work has recently been revived, after a gap of over a decade, on the production of malaria antigens as potential vaccine candidates in the milk of transgenic goats. In the short term, the aim is to harvest and purify the protein from the milk for vaccine production and testing, but the long term vision is to deliver the vaccine orally in the milk. However, there are many technical, regulatory and policy challenges to be faced before this goal could be achieved.

7. Recent focus has been more on the use of transgenic plants as an alternative to traditional methods for vaccine production. The development of efficient plant-based expression strategies and new concepts for the purification of recombinant proteins has facilitated progress in this area. Antigens from several human and veterinary pathogens have been expressed in transgenic plants, e.g. rabies, hepatitis B, measles, avian influenza and anthrax. Plant-based bioreactor systems offer several potential advantages over other production methods, with relatively low costs of cultivation, scale up and maintenance for

[7] Cywes-Bentley et al. (2013) Antibody to a conserved antigenic target is protective against diverse prokaryotic and eukaryotic pathogens. PNAS 2013 : 1303573110v1-201303573.

vaccine manufacture. It is also suggested that the production of edible subunit-based recombinant vaccine proteins in the leaves, seeds or fruits would allow products to be easily stored and transported with limited refrigeration, and would provide a means of oral administration that would need less effort and reduce the required technical training of medical and veterinary personnel. But before plant-based edible vaccines could become a reality, many concerns about technical, regulatory and policy issues need to be addressed.[8] An alternative approach is to use plant-based production systems to generate recombinant proteins that are then extracted and purified before administration by traditional methods. This requires additional manufacturing time and cost.

8. One example is the potential use of tobacco plants as an alternative to making influenza vaccines in embryonated eggs; a plant-based expression system could provide greater speed and capacity than other recombinant technologies at a comparatively lower cost. This is particularly important to meet the surge capacity required for the production of pandemic vaccines. A vaccine candidate based on transient expression of the recombinant protein antigen in the leaves of the tobacco plant, which is then extracted and purified to obtain clinical grade material, is in clinical trials.[9]

9. There have been many reports on studies on the use of transgenic plants for the production of veterinary vaccines, based on either edible or purified systems. One recent example is the development of transgenic alfalfa plants expressing a recombinant antigen that could induce neutralising antibodies to bovine viral diarrhoea virus (BVDV). A standardised approach for a low cost purification process was also devised. This work provided data to support the feasibility of producing reliable vaccines in plants as an inexpensive alternative to traditional fermentation systems.[10]

IV. Vaccine delivery

10. Recent developments in vaccine delivery have drawn on advances in nanotechnology and microencapsulation. Non-parenteral routes of administration, such as intranasal, aerosol, oral and transcutaneous are also being exploited. For example, nano-vesicles have been shown to be capable of encapsulating antigens thus enhancing their intracellular delivery and increasing their temperature stability, which means that they require less care in handling and storage. Nano-vesicle technology is also suitable for a variety of non-parenteral routes of administration and has the additional benefit of helping to boost the host response to the vaccine.[11]

11. A recent report has provided support for a novel vaccination concept that uses gold nanoparticles as a delivery vehicle for presentation of viral antigens to induce a protective immune response. Gold nanorods of a similar size and shape to the virus particles were coated with the major protective antigen, a surface protein from the virus. This construct was found to mimic the live virus by delivering the protein to antigen presenting cells, specialist immune cells that are key players in induction of the immune response, and

[8] Gunn et al. (2012) Using transgenic plants as bioreactors to produce edible vaccines. Journal of Biotech Research 4: 92-99.

[9] Vezina et al. (2011) Plants as an Innovative and Accelerated Vaccine-Manufacturing Solution. Supplement to BioPharm International May 2011 s27-s30.

[10] Aguirreburualde et al. (2013) Efficacy of a BVDV subunit vaccine produced in alfalfa transgenic plants. Veterinary Immunology and Immunopathology 151:315-324 doi:10.1016/j.vetimm.2012.12.004.

[11] Pardakhty, A. & Moazeni, E. (2013) Nano-niosomes in drug, vaccine and gene delivery: a rapid overview. Nanomedicine Journal 1: 1-13.

allowing induction of an immune response in human cells in the laboratory. Further work is required to determine if such vaccines would work in vivo; however this platform could potentially be used to develop vaccine candidates for other viruses as well as larger pathogens such as bacteria and fungi.[12]

12. The development of nanotechnology-based skin patches has offered a potential alternative for vaccine delivery to overcome some of the disadvantages of needles and syringes. These small patches contain many tiny projections which are coated with the vaccine in dried form. When applied to the skin the projections become wet and the vaccine is released in the narrow layer just beneath the skin surface which contains a high density of antigen presenting cells required to generate a protective immune response. This means that a lower dose of vaccine is required. In addition, unlike traditional vaccines, the dry formulation of the patch is expected to minimise or eliminate the need for refrigeration during storage and transport. It does not need to be administered by trained medical staff, and is anticipated to avoid needle-phobia, needle-stick injuries, cross-contamination and costs associated with the disposal of the delivery device and packaging. All of these attributes have the potential to reduce costs considerably. Initial studies have been reported on influenza vaccine, but it is anticipated that nanopatches could be suitable for delivery of many vaccines. FF[13] However, there are still issues to be addressed and it could be many years before this technology could be ready for the market. Trials will be taking place in both developed and developing countries with the aim of enabling both to take advantage of the technology at the same time. FF[14]

13. Another means that has been proposed to address the limitations of the refrigeration requirement for distribution and storage is the stabilisation of vaccines in silk protein. Cold chain requirements represent a major economic and logistical burden, particularly in low resource settings where reaching the relevant populations may mean transporting the vaccines to areas where electricity and refrigeration is limited. Failures in the cold chain result in costly waste of vaccines and degradation can result in the delivery of ineffective doses, which may not be apparent at the time. The structure and properties of silk make it highly thermodynamically stable and mechanically robust, and it contains nanoscale pockets that can immobilise bioactive molecules and improve their stability. Encapsulation of vaccines in silk matrices has been evaluated with the MMR vaccine, demonstrating that the matrices were capable of stabilising labile vaccines for more than six months over a range of tropical temperatures. Thus this has been suggested as a transformative approach to the cold chain system and a feasible path forward to provide more efficient and widespread distribution of vaccines and other labile therapeutics throughout the world.[15]

V. Implications

14. As with many fields in the life sciences, advances in vaccine development have the potential for uses contrary to the provisions of the Convention. Knowledge gained through research on the pathogenicity of the disease agent and the host immune response to assist

[12] Stone et al (2013) Gold nanorod vaccine for respiratory syncytial virus. Nanotechnology 24: 295102 doi:10.1088/0957-4484/24/29/295102.

[13] Fernando et al. (2010) Potent Immunity to Low Doses of Influenza Vaccine by Probabilistic Guided Micro-Targeted Skin Delivery in a Mouse Model. PLoS ONE 5(4): e10266 doi:10.1371/journal.pone.0010266.

[14] http://www.guardian.co.uk/global-development/poverty-matters/2012/dec/26/vaccine-disease-papua-new-guinea.

[15] Zhang et al. (2012) Stabilization of vaccines and antibiotics in silk and eliminating the cold chain. PNAS 2012 : doi:10.1073/pnas.1206210109.

vaccine design could also be exploited for harmful purposes, for example, to design novel BW agents or alter the characteristics of existing agents to increase their suitability for BW use. Advances in technologies that make vaccine production simpler, faster, cheaper and more efficient also have the potential to be used for BW agent production. Likewise concepts developed to deliver vaccines to specific cell types could also be used to design delivery platforms for harmful materials. This potential underlines the critical importance of early consideration of all the implications of relevant scientific and technological advances, including the possible need to develop appropriate strategies for oversight and governance, including possible changes to existing safety and product licensing regulatory frameworks. Such measures would need to be designed to prevent prohibited activities without having adverse effects on legitimate activities, and as such would also be relevant for discussion under the Standing Agenda Item on strengthening national implementation.

15. As discussed in the UK Working Paper, 'Confidence-building Measures: next steps to enable fuller participation' submitted to this Meeting of Experts, scientific and technological developments in vaccine design and production need to be taken into account in review of the CBM process. In particular, development of vaccines that do not need containment facilities for production, or emergence of new production methods that are not-based on traditional fermentation technologies are relevant to discussions on which types of facilities will be of most interest to CBMs in future. Such developments would also have a direct bearing on any future work on the design of possible verification and compliance measures for the BTWC. This would be particularly relevant for any declaration regimes that might be deemed necessary.

16. Advances in vaccine development have wide reaching potential benefits for the mitigation of infectious diseases and are highly relevant to the BTWC. Wider availability and timely administration of vaccines will reduce the likelihood of effective and extensive BW use and will be a key factor in developing a global response to infectious disease outbreaks, whether natural, accidental or deliberate. Such capabilities would be important in the implementation of Article VII, in providing assistance to any State Party exposed to danger as a result of a violation of the Convention.[16] The development of cheaper and more readily available vaccines is a significant issue for activities related to Article X, and for the wide range of other international initiatives for the development and application of scientific advances for the global prevention of disease.

17. In particular, research and development that addresses issues of specific relevance to developing country needs has made some significant advances. The design of vaccines that elicit broader spectrum immunity, or that can be designed, constructed and produced rapidly in response to an emerging threat, or that can be produced without biological containment and in geographically dispersed facilities, could significantly enhance the capability for provision of effective, lower cost, rapidly manufactured and widely accessible vaccines. Advances that avoid the need for cold chain handling and for administration by trained medical or veterinary personnel also reduce costs and logistical burden, and have the potential to help get vaccines to where they are needed most. However there are still many technical hurdles to overcome, and there may be situations where vaccines are not currently the appropriate solution; other medical countermeasures such as antibiotics or antiviral drugs may then be the preferred approach. In addition there are fundamental challenges in securing global access to existing and new vaccines, which a range of international initiatives endeavour to address. Global strategies to advocate vaccine development and use are required to overcome lack of uptake for some vaccines.

[16] The Intersessional Programme will address how to strengthen implementation of Article VII in 2014 and 2015; the UK will contribute further during those discussions.

Such aspects are also relevant for discussion under the Standing Agenda Item on cooperation and assistance.

VI. Conclusion

18. The implications of the examples of scientific and technological advances in vaccine development raised in this paper highlight the need to continue to review regularly advances in scientific fields of relevance to the Convention. The pace and complexity of the many developments underlines the utility of 'bringing in more voices' to the intersessional programme to benefit from the knowledge and expertise of those more closely involved in relevant areas. This may assist us in considering fully the implications across all aspects of the Convention and assessing practical consequences and actions, both short and long-term.