PLANT-MADE VACCINES – THE PAST, PRESENT AND FUTURE

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Summary

After 16 years of research, the world's first regulatory approval for a plant-made vaccine for veterinary purposes occurred in early 2006 and marked the possible transition of plant-made vaccine research to plant-made vaccine development. The licensure of the plant-made, Newcastle Disease Virus vaccine demonstrated the technology has technical and industrial feasibility for application with animals. However careful consideration of directions now taken is needed to ensure advancement of the technology in fields other than academia and perhaps towards plant-made vaccines for humans. Fields of particular importance include plant expression systems, downstream processing, intellectual property, freedom to operate, regulations and commercial feasibility.

1. Introduction

Plant-made vaccines were first described in a patent application published in 1990, and based on the first demonstration at Washington University that an antigen could be produced in whole plants. Since then extensive research has been performed in this field by a growing number of plant biology and medical researchers. The ability to produce a vaccine in plant material that could be delivered by feeding would have tremendous value in animal and human health. The technology is attractive, particularly as a health strategy for developing countries, due to possibility of low cost production and needle-free immunisation. However in the intervening years only one plant-made vaccine has

been commercially licensed – a vaccine to protect poultry from Newcastle Disease Virus (NDV). We describe the general technology, historical development and possible future of plant-made vaccines.

2. Traditional Vaccines

Vaccination has travelled a long road since the contents of smallpox pustules (variolous material) were used to inoculate individuals. Vaccination as we know it was founded on the work of Jenner and Pasteur. Both men used weakened (attenuated) forms of a pathogen to decrease mortality of particular diseases. Jenner decreased mortality of small pox by using a naturally attenuated virus, cowpox, while Pasteur worked with synthetically weakened forms of rabies and anthrax. Together their work revolutionised infectious disease prevention. There are three types of traditional vaccines: inactivated vaccines; live attenuated vaccines and toxoids. Inactivated vaccines consist of killed, previously virulent pathogens and include vaccines against influenza, hepatitis A, and cholera.

Live attenuated vaccines consist of live organisms that have had their virulent properties reduced. Examples include polio, chicken pox, measles, mumps and rubella. Toxoids are inactivated forms of poisons or toxins produced by a pathogen. They are usually chemically inactivated using formalin and are used as the basis for vaccines against tetanus and diphtheria. Vaccines revolutionised medicine and ultimately lead to the eradication of small pox and near eradication of polio. Although traditional vaccines are effective, the complex immune responses invoked against the whole pathogen may lead to adverse responses in a small number of instances and if not processed correctly they may actually cause disease in immune compromised, young or elderly individuals. Improved knowledge about pathogens and pathogenicity has lead to a new generation of vaccines that have become the preferred means of immunisation with decreased chance of adverse outcomes.

3. New Generation Vaccines

New technologies such as recombinant DNA technology have been used to improve traditional or develop new vaccines against disease. The resulting vaccines have been called new or second generation vaccines. There are two types of new generation vaccines being used: (i) Conjugate vaccines that link isolated proteins or toxins from a pathogen that are recognised by immature immune systems to the outer coats of the disease-causing bacteria. This enables a young immune system to respond and defend against the disease agent. An example of a conjugate vaccine is one that protects against a type of bacterial meningitis caused by *Haemophilus influenzae* type b (Hib). (ii) Subunit vaccines contain pathogen fragments, or subunits, that are unable to cause disease but are able to induce protective immune responses.

The production and purification of these antigens may be time consuming and more expensive compared to traditional vaccines. However the reduced exposure to the pathogen decreases the patient complication rate. An example of a subunit vaccine is the hepatitis B vaccine that consists of the surface protein of the virus produced by recombinant yeast.

4. Plant-Made Vaccines

Plant-made vaccines are a type of subunit vaccine where the reactor is a plant or plant cell. The vaccine may be delivered in plant tissues, or through a purified or partially purified extract. Plant-made vaccines may use recombinant plant viruses or stable transgenic plant lines. Although plant viruses have been successful at transiently expressing large amounts of protective fragments in plants, this review focuses on stable transgenic plant or plant cell lines that are capable of passing on the gene for the protective fragment to the next generation. Producing a plant-made vaccine begins by selecting a suitable protein fragment or antigen. The corresponding gene of interest is cloned into an expression cassette that contains plant regulatory sequences capable of driving gene expression and showing the gene's end. This cassette is then used in plant transformation. Many techniques have been used to transform plant cells, however *Agrobacterium*-mediated transformation is usually preferred for transformation of the plant cell nucleus. This is because of the low frequency of insertions, and the low number of transgene copies inserted into the host genome at a single insertion point. Both of these characteristics reduce the chance of transgene silencing, a phenomenon responsible for decreased or no expression of the transgene and possibly a related homologue in the host genome. *Agrobacterium* is a plant pathogen that in the process of infection, transfers a segment of its DNA (T-DNA) into the genome of its host. Molecular biologists have taken advantage of this process to transfer a gene of interest, in a plant expression cassette, into plant genomes. Transfer of the T-DNA from the bacterium into the host's genome occurs upon incubation of the transgenic *Agrobacterium* with plant materials. During tissue culture, transformed cells are selected using a marker or resistance gene and regenerated into transgenic plants or multiplied into plant cell lines. The time taken to regenerate a transgenic line is species dependent and ranges from 6 weeks to 18 months. Elite plant lines for further development are selected based on the protective fragment authenticity to the native form and concentration in plant tissues or cells. The elite lines are amplified either in the greenhouse or fermentation reactors (plant cell cultures), fully characterised and then tested for immunogenicity in animal trials.

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Bibliography

Curtiss, R. and G. A. Cardineau (1990). Oral immunization by transgenic plants. World Patent Application. USA, Washington University. [This is the foundational patent for plant-made vaccines].

Kirk, D. D. and S. R. Webb (2005). The next 15 years: taking plant-made vaccines beyond proof of concept. Immunology and Cell Biology 83(3): 248-256. [A timely reference raising issues of research management and strategic development of plant-made vaccines].

Kryder, R. D., S. P. Kowalski, et al. (2000). The Intellectual and technical Property Components of Pro-vitamin A Rice (Golden-RiceTM): A Preliminary Freedom-to-operate Review. Ithaca, NY: ISAAA. [This reference provides insight into the intellectual property landscape of transgenic plants.].

Mihaliak, C. A., S. R. Webb, et al. (2005). Development of plant cell produced vaccines for animal health applications. Proceedings of US Animal Health Association, Greensboro, N.C.

Rigano, M. M. and A. M. Walmsley (2005). Expression systems and developments in plant-made vaccines. Immunology and Cell Biology 83: 271-277. [This work provides an indepth discussion on expression systems for plant-made vaccines and perceived problems for the technology].

Robert, J. S. and D. D. Kirk (2006). Ethics, biotechnology, and global health: the development of vaccines in transgenic plants. Am J Bioeth 6(4): W29-41. [This reference gives a comprehensive listing of ethical issues associated with plant-made vaccines including a chronology of early developmental milestones.].

Walmsley, A. M. and C. J. Arntzen (2000). Plants for delivery of edible vaccines. Current Opinion in Biotechnology 11: 126-129. [This represents a general description of the plant-made vaccine technology].

Walmsley, A. M. and C. J. Arntzen (2003). Plant cell factories and mucosal vaccines. Curr Opin Biotechnol 14(2): 145-50. [This review provides an update of Walmsley and Arntzen (2000)].

Biographical Sketches

Dr Amanda Walmsley has been researching in the field of plant-made vaccines for the past 8 years and is proud to be part of the team responsible for getting the world's first plant-made vaccine to licensure. She received her Ph.D. in botany in 1998 from the University of Queensland, Australia and researched as a post-doctoral fellow at the Queensland Agricultural Biotechnology Centre in Brisbane, Australia. Dr Walmsley then moved to the Boyce Thompson Institute for Plant Research, Inc., Cornell University, USA to research as a visiting scholar before accepting a position as an Assistant Research Professor at Arizona State University in 2001. Dr Walmsley joined the School of Biological Sciences at Monash University as a Monash Fellow in January of 2006.

Dr Dwayne Kirk has worked in the field of molecular farming for the past 10 years. He was Project Manager at the Boyce Thompson Institute for Plant Reseach (Ithaca, New York, USA) for a strategic alliance with Dow AgroSciences and is a co-inventor on patent applications supporting the world's first commercial vaccine produced by transgenic plant cells. He has assisted in the conduct of two human trials with plant-made vaccines and has published scientific papers describing many aspects of plant-made vaccine development including downstream processing, strategic planning and ethical considerations. Dwayne completed his PhD at Arizona State University and combined aspects of process development with law and business to evaluate commercial feasibility of this technology. After spending most of the past decade in the US he returned to Australia in 2006 as a Research Fellow at Monash University (Melbourne, Australia).