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Plant-based vaccines for animal health

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Summary

Plant-based vaccines are recombinant protein subunit vaccines. Ideally, the choice of plant species used to produce the selected antigen should allow for oral delivery in the form of an edible vaccine. These vaccines are well suited to combat diseases where there is a clear antigen candidate, and where the costs of production or delivery for any current vaccine are prohibitive. Several academic and industrial research groups are currently investigating the use of plant-based vaccines in both humans and animals. To date, the most advanced human vaccine projects have successfully completed phase I clinical trials, and animal vaccine projects have given promising data in early phase trials targeting specific animal species. In this article the advantages offered by plant-based vaccines will be presented, progress on the most advanced vaccine candidates will be summarised, and the path ahead will be outlined. Although the focus of this paper is on the application of plant-based vaccines in the field of animal health, principally their use in domestic livestock, examples of the use of plant-based vaccines in the field of human health will also be discussed.

Keywords

Animal health – Animal vaccine – Edible vaccine – Oral delivery – Oral vaccine – Plant-based vaccine – Subunit vaccine – Vaccine bulk-up – Vaccine production – Vaccine storage.

Introduction

The administration of vaccines is by far the most humane and cost effective method of combating the spread of diseases. Successful vaccination programmes lead to far fewer individuals ever showing symptoms of disease, thus reducing the need for costly treatment procedures. In cases where efficacious vaccines have been broadly implemented there have been dramatic impacts on life-threatening diseases, most notably in the field of human health, e.g. the eradication of smallpox in the field and the greatly reduced threat of poliomyelitis. Similarly, in the field of animal health there are numerous examples of vaccines that greatly reduce mortality (e.g. vaccines directed against foot and mouth disease). For vaccines developed for domestic livestock the protection afforded by an efficacious vaccine not only removes the need for the administration of treatments, but also guards against the economically damaging consequences of disease (e.g. reduced weight gain and productivity).

Despite the obvious advantages of vaccination, for many diseases there are still severe limitations with currently available vaccines. In some cases, such as with acquired immune deficiency syndrome, there is not yet any clear vaccine candidate, while in many others, such as with most cholera vaccines, efficacy is partial. Although efficacy relates largely to the vaccine material, the mode of delivery should also be chosen carefully so as to induce the best possible response. Most vaccines are delivered by injection, even though, as in the case of influenza, the best means of protection is likely to be stimulating a mucosal response by delivering the vaccine directly to the site of pathogen invasion (i.e. the mucosal barrier). Delivery via injection also greatly raises the overall vaccine cost as disposable materials and personnel trained in vaccine administration are required. The cost of employing personnel trained in

animal handling procedures must also be considered. Other efficacious vaccines engender safety concerns, particularly those using live delivery vehicles such as modified live viruses, as is the case with the currently available smallpox vaccine. For other vaccines, logistical issues of production costs, bulk-up capacity (i.e. the practicality of production being able to meet demand and the speed with which this can occur), and stable storage and distribution may limit vaccine uptake. Limited production capacity is currently as issue associated with the influenza vaccine in the United States of America. For injected vaccines in particular, the maintenance of a cold chain through storage and distribution is generally essential, thus further adding to costs.

Plant-based vaccines offer potential remedies to several of the above drawbacks of conventional vaccines (28). Since plant-based vaccines consist of protein subunits, a good candidate antigen must first be identified in order to develop the vaccine. If this exists, there are several potential advantages of using plant technology for the production of vaccines; most notably, the overall costs can be greatly reduced compared with competing systems. This is in part due to the relatively low facility and maintenance costs required for large-scale plant production, but is mainly due to the cost advantages afforded by the oral delivery of antigen. Edible plant-based vaccines eliminate the need for disposable injection materials and trained personnel to administer the injections. Moreover, farmed animals do not need to be gathered to receive an injection; an edible product can conveniently be added to their regular feed. Oral vaccine administration also avoids carcass scarring caused by injections. Since very large quantities of antigen can be produced by plant systems, the issue of limited capacity should not arise. Furthermore, this greatly increased production capacity means that oral doses of antigen can be much greater than those previously considered, thus facilitating immune response. The oral delivery of large amounts of antigen will induce a serum immune response and, unlike antigens that are injected, stimulate a substantial mucosal immune response. This makes plant-based vaccines particularly suitable for combating intestinal pathogens and, due to the generalised nature of mucosal immunity, pathogens invading other mucosal sites. Certain types of plant material, such as grains, store proteins in a dehydrated and stable condition for years at ambient temperatures, thus allowing for inexpensive storage and distribution. In addition, since plant-based vaccines are subunit vaccines, there tend to be fewer safety concerns than when considering live delivery vehicles. This paper provides examples of plant-based vaccine candidates under development that can overcome some of the limitations of current vaccines. Novel plant-based vaccines targeting diseases for which conventional vaccines do not exist will also be discussed.

Developing a plant-based vaccine

Plant-based vaccines are subunit vaccines in which the antigen of interest is produced in plant tissues. As with all recombinant protein subunit vaccines, a prerequisite for vaccine development is the identification of a suitable antigen with a known ability to confer protection (or at least a high probability of doing so). The antigen, or antigens, must then be expressed at a sufficiently high level in the chosen plant production species to allow for the practical oral delivery of a sufficient antigen dose to induce protection. A plant species should be chosen that has optimum antigen expression, allows for cost-effective vaccine production, and can be manufactured into a practical form for oral delivery. Once the selected antigen has been expressed at a high level in the plant, suitable processing technologies must be applied to yield the final form of the vaccine for delivery. The vaccine candidate must then be assessed through appropriate animal safety and efficacy trials. If the vaccine is going to be used in humans, it will then enter clinical trials. Long-term stability during storage and distribution must also be assessed.

Selection of an antigen target

The starting point for antigen expression is to select a suitable protein target. Ideally, when an efficacious protein subunit vaccine already exists and an alternative plant-based oral option is being considered for reasons of cost, the established vaccine antigen can be introduced into a plant expression system. In the absence of a currently used antigen, a novel candidate can be selected by focusing on known or predicted toxin subunits or surface antigens associated with the pathogen of interest. When dealing with a poorly characterised pathogen, a genomics or proteomics approach might first be used to identify antigen candidates that possess promising characteristics (e.g. cell surface virulence factors).

Selection of a plant expression host

After selecting an appropriate antigen, a suitable plant expression host must be chosen. There are several issues to be considered when choosing an expression host. Perhaps the most critical is what form the candidate vaccine will take for delivery. Foreign proteins can be expressed in fresh tissue, such as mature plant leaves (18), germinating seedlings (23) or the fronds of the aquatic plant duckweed (24). Alternatively, the selected protein can be expressed in dry tissue, such as the seeds of cereals (25). While grains can stably store proteins for years, expression in fresh tissues requires immediate processing to a form in which the foreign protein will not be degraded. A further option

is to secrete the expressed protein into the surrounding medium. One possibility is to maintain the plants using hydroponics, express the selected protein in the roots, and direct it for secretion into the surrounding aqueous medium (water plus nutrients for plant growth). This approach has been applied to greenhouse-grown tobacco (5). A second possibility is to express the foreign protein in a plant tissue culture system, such as those that have been developed for tobacco (11) and rice (33).

Several different plant species have been experimented with for the purpose of expressing antigens at high levels. In academia the most common plant chosen as an expression host is tobacco because it is relatively easy to transform and successful expression can be assessed quickly. However, in industry more focus has been placed on plant species, such as cereals, that:

– offer long-term stability of the expressed protein

– are cost effective

– rapidly produce large volumes of the desired product

– can be easily processed into a deliverable form for oral vaccination.

Generation of transgenic plant material

Antigen expression in fresh leaf tissue can be achieved by a variety of means. Nuclear transformation produces transgenic plants with deoxyribonucleic acid that encodes the foreign protein and is integrated into the plant's genome such that it is stably inherited from generation to generation. Nuclear transfer methods are widely applied to several major crop species and species used principally for experimentation, such as *Arabidopsis thaliana*. A second approach for achieving expression in leaf tissue is the transformation of chloroplasts that have their own genome. Although high levels of antigen expression have been attained in chloroplasts (10), this technology is currently limited to tobacco. In this case, transgenic plants must be maintained through the maternal line, since pollen is essentially free of plastids, of which chloroplasts are a form. The expression of antigens in leaves can also be achieved by infecting plants with an engineered plant virus carrying sequences encoding for the desired protein. The virus invades plant leaf tissues and uses the cellular machinery of the plant to produce the foreign protein. In this approach the germ line of the host plant is not affected, so viral infection of further plants must be undertaken for each production cycle. Like chloroplast transformation this approach has been applied mostly to tobacco (39), but has also been used in black-eyed beans (9) and spinach (40).

Expression in the seeds of cereals is achieved by nuclear transformation (antigen candidates have been expressed in maize [25]). Nuclear transformation is also used to generate transgenic plants that are grown using hydroponics or plant tissues that are maintained in tissue culture: in each case the antigen is secreted. Figure 1 shows the relationship between the plant tissue chosen for antigen expression and the available technologies to generate the transgenic material.

Achievement of high levels of antigen expression in plants

Expressing the chosen antigen at a sufficiently high level in the selected plant host tissue is vital in developing a viable plant-based vaccine. The expression level must be sufficient to allow for oral delivery of the required antigen dose, in an appropriately processed form of plant tissue, to the patient or target animal. A great amount of effort continues to be focused on achieving high levels of antigen expression since for some antigens very high doses may be required to provide protection through oral delivery.

Several approaches have been used to raise the level of antigen expression, such as the use of strong plant promoters (7, 25) and untranslated leader sequences (22). For some of the selected antigens, target sequences have been applied to a range of organelles to determine which sub-cellular environment produces the greatest amount of stable protein (22, 27). In fresh leaf tissue an alternative way to direct accumulation of the antigen in the

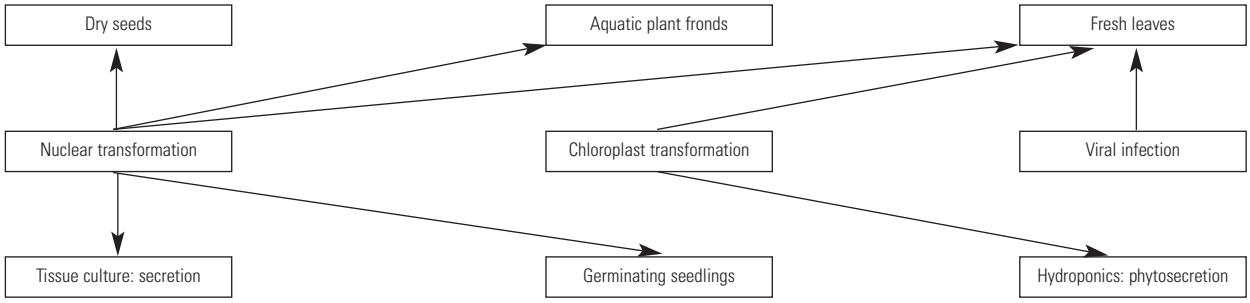

Fig. 1
Techniques used to produce transgenic plants and the various plant materials to which each method can be applied

chloroplast is to transform this organelle. Since each cell can contain up to 10,000 plastid genomes, transformed chloroplasts will produce a very high number of copies of the trangene without inducing the gene silencing phenomena often associated with a high copy number of transgenic plants (20). The expression of antigens in chloroplasts has reached levels comparable to the best performing transgenic plants generated by nuclear transformation (10).

A further technique that is used to achieve high levels of protein expression in plants is to introduce the transgene into a germplasm that is well suited for high levels of protein production. This approach is possible with genetically well-characterised species, such as maize, in which different lines and mutants express varying levels of the major seed proteins (8). These lines may not be suitable for transformation; necessitating introduction of the transgene into a receptive line; followed by introgression of the sequence into the desired line through a suitable breeding programme.

Other approaches typically applied today to microorganism expression systems can also be applied to plants. For example, an epitope of the selected foreign protein can be expressed as a fusion protein by combining it with a well-characterised and stable carrier protein.

Structure of the expressed antigen

In addition to the expression level, the form of the plant-expressed antigen is also very important in generating an appropriate immune response (i.e. the antigen should be full-length and appropriately folded). The expression of full-length antigens in plant tissues, including those over 100 kD in size (13), has been described in many reports. In the case of multi-subunit antigens, where formation of the quaternary structure is important for inducing a full immune response, the subunits should be properly associated in the plant tissue. For example, the receptor binding subunits of the heat-labile toxins of *Escherichia coli* and *Vibrio cholerae* assemble into pentamers when produced by their respective pathogens. The formation of pentamers is necessary to enable binding of the receptors to the surface of the gut, which is required to induce a strong immune response. The expression and formation of these subunits into pentamers in plant tissues has been reported previously (1, 12, 26).

Many proteins undergo some form of post-translational modification, such as the addition of carbohydrates, lipids or other functional groups. Most significantly, glycosylation can greatly alter the surface of an antigen, thus affecting the shape of the antigen presented to the immune system. Many viral surface proteins are heavily glycosylated, whereas bacterial proteins are non-glycosylated. A suitable level of glycosylation can be achieved by selecting an appropriate plant expression technology. For example, if glycosylation is preferred the antigen should be directed to the endomembrane system and possibly secreted. Alternatively, glycosylation can be avoided by expressing the antigen in the cytoplasm. However, if a membrane environment is required for high levels of expression the addition of carbohydrates can be blocked by mutation of the predicted glycosylation sites. Since plastids do not glycosylate proteins, glycosylation can also be prevented by using chloroplast transformation technology to generate the transgenic plants.

As indicated by their mobility following electrophoresis, several viral antigens have been expressed in plants with approximately the same amounts of glycosylation, although the structures were not determined (13). While plants and mammals have similar patterns of glycosylation, there are some differences: plant glycans use an α-1, 3 fucose linkage rather than an α-1, 6 fucose linkage, have β-1, 2 xylose linkages, and lack sialic acid moieties. Research programmes are underway to knock out plant specific glycosylation enzymes (35) and introduce mammalian glycosylation enzymes into the plants (4). In this way the plant glycosylation machinery can potentially be engineered to modify foreign proteins in a manner indistinguishable from that of their source organism.

Antigen purification and options for delivery

Proteins have been purified from plants and are already being produced on a large-scale for sale in fine chemical markets, most notably with the first large-scale commercial product, trypsin produced in corn (37). Purification is the most obvious next step for proteins expressed in a plant tissue culture fermentation system or in plants grown under hydroponics, where the protein is secreted from the roots. The protein purification approach is possible for candidate vaccine antigens expressed in recombinant plants, in which case a purified antigen could be delivered by any chosen means, including injection. However, this approach is likely to be prohibitively expensive for animal vaccines, and to properly realise the economic potential of plant-based vaccines it is necessary to pursue an oral delivery option based on a native or processed form of the plant material.

In considering animal vaccines, some plant expression hosts yield material that can potentially be harvested, stored as it is, and directly delivered to the target species. For example, in the case of a vaccine candidate for domestic livestock, the antigen can be expressed in the seed tissue of a cereal. The grain can then be harvested, packaged and stably stored for extensive periods of time without further processing. Subsequently, the grain can be distributed and delivered directly to the animals. In this

case, handling costs are largely restricted to those required for identity preservation, ambient temperature storage and distribution. A candidate antigen for swine has been expressed in maize seed at a sufficiently high level to allow for the desired dose of the antigen to be administered in the form of unprocessed grain (17, 26). In this case, the grain can be added to the standard feed of the animals. Although expression of a foreign protein typically varies from plant to plant, and within grains even from seed to seed, expression of an antigen across a field harvest of a developed transgenic line is uniform, thus facilitating even dosing, even in the case of delivering unprocessed grain as an edible vaccine.

In contrast, many plant tissues, notably fresh leaves, cannot be administered in their unprocessed form and require some level of processing. Leaf material must be processed upon harvest in such a way as to allow storage of the antigen in a reliably stable state. Furthermore, the processed material must be palatable enough to allow for oral delivery. While this is the case for some crops, such as alfalfa, it has not been possible for tobacco: the species that is most widely used as an expression host. The amount and concentration of antigen expressed in fruit (e.g. tomatoes) tend to vary between individual fruits, so they must be processed to homogenise the foreign protein; this should immediately follow harvest to ensure protein stability. Tomatoes expressing a fusion protein, such as the receptor binding subunit of the heat-labile toxin of *E. coli*, are processed by freeze-drying, followed by powdering and pooling. In this form the antigen is concentrated, homogeneous, and stable, at least when maintained in dry, cold storage (34). Certain antigens have also been expressed in vegetable tubers, these being more suited to long-term storage without immediate processing. For example hepatitis B surface antigen has been expressed in potato tubers (22). However, the variation in expression between tubers is such that processing would still be necessary prior to delivery in order to produce a product with a uniform antigen dose.

In addition to the option of direct delivery, the seeds of cereals also offer several relatively inexpensive processing options. The grains can be processed into a variety of forms, such as pellets, using established technologies. These processes can be adapted to avoid very high temperatures and pressures, therefore ensuring that the expressed antigen is not degraded and does not lose functionality (26). Although grains can be delivered to domestic livestock in their harvested form, processing does have some advantages, such as increasing the concentration of the antigen and improving the stability and homogeneity of the antigen. Through the processing procedure, the antigen can be concentrated to reduce the amount of material needed to deliver a prescribed antigen dose. For example, the fraction of the seed expressing the antigen can be separated from other seed tissues. This is relevant when a tissue specific promoter is used to express the foreign protein specifically in the germ or the endosperm of the seed. These tissue types can be easily separated by milling procedures. The germ or endosperm can then be processed into a form that is suitable for delivery. The germ may also be defatted to further concentrate the antigen and prevent the material from becoming rancid during storage. Certain processed forms of the grain are very stable during long-term storage, and homogeneity will typically improve with large-scale processing. Further advantages offered by processing include the creation of a product that is more easily stored, packaged, distributed and delivered.

Processing of plant material to generate a palatable form of the vaccine that contains a highly concentrated antigen is particularly important for human vaccine candidates: palatability and delivery of a small amount of material are important issues. The preferred methods of processing available for various plant tissue types expressing candidate vaccine antigens are shown in Figure 2. Whether the plant tissue is processed or not, oral delivery of antigens encased in plant material does appear to result in

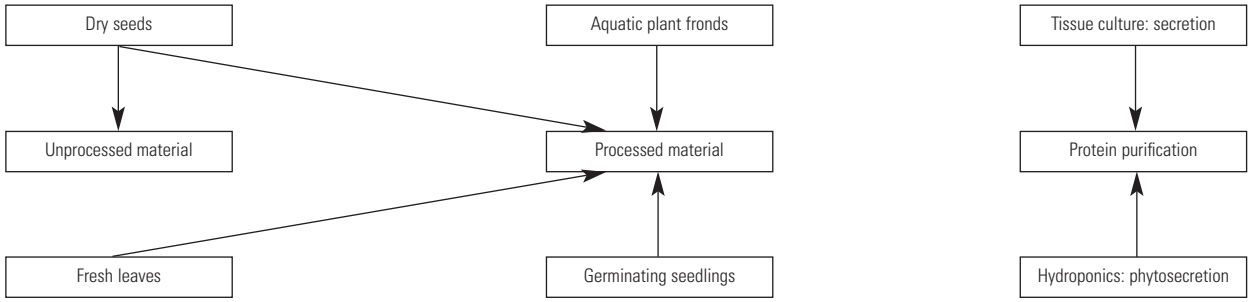

Fig. 2
Options for preparing vaccine material from the various plant tissue types expressing candidate vaccine antigens

improved immune responses compared to the oral delivery of pure antigens (3). Presumably, this reflects a partial protection and gradual release of the antigen during its passage through the digestive tract of the target species. Encapsulation of the expressed protein is, thus, considered to be a characteristic of plant-based vaccines.

Progress in research and development

There are now a large number of candidate vaccine antigens that are expressed in plant systems (See Streatfield and Howard [29] for a comprehensive listing). The rate at which new candidates are being expressed in plants, or previously selected candidates expressed in alternative plant systems has lead to a rapidly increasing number of antigen expression studies in plants. The majority of these reports investigate human vaccine candidates, but there are also an increasing number of studies directed at animal vaccination: to improve animal health and/or combat human health threats.

Most projects studying plant-based vaccine candidates, whether targeted at humans or animals, have not progressed beyond the initial phase of expressing the selected antigen in a plant system and testing for an immune response following injection of the transgenic plant extract into a laboratory test species (typically the mouse). Although most studies report antigen expression and mouse injection studies typically result in the generation of antigen specific antibodies, many factors limit further development of the vaccine candidate. For example, the chosen plant species (which is often tobacco) is not suitable for use as an oral vaccine, or the level of antigen expression is orders of magnitude below those required for oral delivery of the required dose. Also, the antigen is often expressed as a peptide that will not stimulate a significant level of protection. Some antigens, especially peptide antigens, may also be expressed in a form that makes it problematic to gain regulatory approval of the vaccine (e.g. fused to a carrier protein that has associated safety concerns).

However, there are several cases in which some of the above limitations have been overcome and the candidate plant-based vaccines have induced immune responses following oral delivery to laboratory animals (reviewed by Streatfield and Howard [29]). In the most advanced cases oral delivery has led to immune responses being observed in the target animal species (including in human subjects in phase I clinical trials [15, 30, 31, 32, 40]). In certain cases, where animal models are available, the oral delivery of a plant-based vaccine has also resulted in protection against disease challenge. In the patent mouse assay, mice fed cholera or travellers' diarrhoea plant-based vaccines were protected against relevant toxins (2, 6, 19, 25). In a notable example of an animal health study using a target animal species, piglets administered an edible plant-based transmissible gastroenteritis virus vaccine showed a high degree of protection when subsequently challenged with the virus (16, 25). The delivery form of the antigen in this case, corn grain, is practical for further vaccine development.

A variety of immune responses have been recorded following oral delivery of plant-based human or animal vaccine candidates. Antigen specific serum immunoglobulins (Ig) G and A have been induced in several studies (29) and some studies have also shown the induction of intestinal mucosal IgG and IgA (2, 19, 25). In phase I human clinical studies, antigen specific IgG and IgA antibody secreting cells have been detected in the peripheral blood indicating the priming of the intestinal mucosal immune system (30, 31, 32). Studies with plant-based vaccines are beginning to look at the immune responses in greater detail. For example, elevated cytokine and cluster of differentiation antigen 4^+ lymphocyte levels were observed when mice were fed potato tubers carrying epitopes of rotavirus enterotoxin and an enterotoxigenic *E. coli* fimbrial antigen fused to cholera toxin sequences (38).

Carrier proteins, such as the receptor binding subunits of enterotoxigenic *E. coli* heat-labile toxin and cholera toxin, and protein adjuvants, such as mutated non-toxic forms of the *E. coli* heat-labile toxin, could be considered for use in plant-based expression systems. When combined with antigens that are unable to induce a strong immune response these molecules would function to boost the immune response. A carrier protein would be fused to the selected antigen, whereas a protein adjuvant would be separately expressed and the plant material expressing the adjuvant mixed with that expressing the antigen. However, addressing safety concerns raised by the use of these carrier and adjuvant proteins would probably prolong the regulatory process required for release of the product.

In certain cases, combining the oral delivery of a plant-based vaccine with an established injection scheme may be sufficient to significantly increase efficacy and/or reduce costs. For example, in a feeding trial targeting transmissible gastroenteritis virus in swine, an initial injection regimen was administered to sows, and subsequently a corn-based vaccine was delivered orally. This resulted in increased levels of neutralising antibodies in the sows' serum and, more significantly, in their colostrum and early milk (17). Since piglets are most vulnerable to disease soon after birth, when they are dependent on the sow's milk, high neutralising antibody levels in the colostrum may be the best way to provide protective immunity.

A further study demonstrating lactogenic immunity has been conducted in mice, where dams were immunised by

oral delivery of a potato tuber-based rotavirus vaccine. Suckling pups were protected against challenge with the virus, indicating that they had acquired passive immunity (38). In a similar study, an epitope of a bovine rotavirus protein expressed in alfalfa was delivered orally to dams and the suckling pups were protected against challenge with the virus (36).

To date, only small-scale feeding studies have been conducted with plant-based vaccines; thus, there is limited data on safety. However, the studies completed so far have not raised any safety concerns. Most notably, no safety concerns have been identified following the testing of several plant-based vaccines in human phase I clinical trials (15, 30, 31, 32, 40) apart from some reactions of nausea in cases where the delivered plant material, raw potatoes, was not palatable to humans (30, 31). Furthermore, since they are recombinant subunit vaccines and are produced in expression systems that are not known to harbour human or target-animal pathogens, plant-based vaccines should be free of disease causing agents.

A potential concern for orally delivered vaccines is the development of oral tolerance. To date, evidence of oral tolerance has not been documented in any plant-based vaccine studies; however, this issue has not yet been rigorously addressed. In all advanced stage clinical trials issues of safety, including that of tolerance, should be assessed. Plant-based vaccines will be assessed for safety in the same way as other oral vaccine candidates.

The path to products

The most advanced plant-based vaccines directed against human pathogens have successfully passed through phase I clinical trials. However, results from the first of these trials were published over six years ago, and there are as yet no reports of phase II clinical trials. This reflects the greatly increased expense of conducting advanced stage trials. Phase I clinical trials were conducted by academic institutions or small biotechnology companies working with a single antigen (although multiple antigens may be necessary for a viable vaccine). In order to progress to later phase clinical trials, particularly beyond phase II, a much greater investment is likely to be necessary than these groups can afford. Rather, the research will need to be undertaken by, or in partnership with, larger pharmaceutical companies that can provide the necessary investment and expertise to guide the most promising projects along the regulatory path towards product approval.

Compared to human vaccines, the regulatory process is greatly simplified for animal vaccines. Swine transmissible gastroenteritis virus vaccine, the most advanced plant-based vaccine candidate for animal health on which data has been published, has shown great potential for further development. In this case, the selected antigen was expressed at a sufficiently high level to allow for oral delivery, and efficacy data appears to be very promising (16, 17, 25). As with phase I human clinical trials, the swine trials were conducted by a small biotechnology company. However, the reduced cost of taking animal vaccine candidates through later stage regulatory trials, compared to that for human vaccines, makes them a more practical commercial venture for small companies. Also, unlike plant-based human vaccine candidates, major animal health companies are showing interest in plant-based vaccines for animals.

There are several issues that will need to be resolved as leading plant-based vaccine candidates enter later stage trials. Definitions will need to be established for master and working seed banks, which will probably be developed from current definitions for master and working cell banks. Furthermore, for each vaccine candidate the type of transgenic plant material used to deliver the antigen will need to be selected. Different delivery forms may vary in efficacy and stability. A suitable dosing regimen will also need to be established for each vaccine candidate. In some cases plant-based oral vaccines may be combined with current vaccine delivery programmes. For example, a plant-based vaccine may be used as a booster following an initial injection. Plant-based vaccines are administered orally as edible-products and, thus, are not prepared or stored under sterile conditions. However, good manufacturing practices must be developed to produce vaccine lots for regulatory trials and subsequent commercialisation.

A regulatory framework has already been established and is being further developed to govern the growth of crops expressing pharmaceuticals, including antigens for vaccine production. Requirements for field production vary somewhat depending on the characteristics of the crop (e.g. whether the plants readily outcross) and the technology used (e.g. whether the plants are transiently or stably transformed by plant viruses). Several strategies can be deployed to contain transgenic crops. There are strict regulations stipulating the required isolation distances between crops expressing pharmaceuticals and other plants of the same species. These distances vary by crop and depend on species-specific characteristics, such as pollen flow and the potential for dispersal of seeds or other plant material. Temporal isolation is another strategy commonly deployed. Here crops expressing pharmaceuticals are planted out of phase with food and feed crops, so guarding against cross pollination. With some species, mutant lines (i.e. male sterile lines) can also be used to further contain the transgenic crop. Using chloroplast transformation technology also greatly reduces the potential for cross-pollination with transgenic material,

since pollen generally do not contain chloroplasts. The planting, harvesting, and transporting of transgenic material are rigorously documented and are accomplished using dedicated equipment that is thoroughly cleaned after each use. After the harvesting of transgenic crops the field sites are monitored for the germination of any transgenic seeds remaining in the field that could give rise to sexually mature plants.

Currently, transgenic crops are planted on very small acreages with the intent of developing high expressing plant lines and generating material for trials. Thus, much of the material generated for this purpose is grown in contained greenhouses. However, economic criteria dictate that for anything but small volume products outdoor plantings are likely to be required.

Promising targets

As for all protein subunit vaccines, a prerequisite for plant-based vaccines is the expression of a distinct antigen that does not mutate rapidly in the wild. Given this condition, the most promising targets for plant-based vaccine may be pathogens that invade the gastrointestinal tract. This is the immediate site of recognition for plant-based vaccines delivered orally, and several vaccine candidates delivered orally have induced mucosal immune responses in the gastrointestinal tract. Pathogens that invade the body via other mucosal surfaces are also good targets for edible plant-based vaccines because the induction of an immune response at one mucosal surface allows priming of other mucosal surfaces.

Plant-based vaccines produced in grains offer the potential for long-term stable storage and the capacity to be bulked-up fairly rapidly at a relatively low cost. Thus, in the field of animal health this technology is well suited for the management of diseases that periodically have major outbreaks, such as foot and mouth disease and rinderpest. In the former case, commonly emerging new strains would mean that the technology used to generate the transgenic material would need to be quickly adaptable. The use of the plant viral approach may meet this criterion.

However, the most obvious advantages of using edible plant-based vaccines for animal health are the relatively low cost of production and, in particular, the delivery method. When considering large-scale vaccine production, plant-based systems can be competitive due to low production facility costs; however, the major saving comes with oral delivery of edible products, since purification and administration costs are significantly reduced. This should make plant-based vaccines highly competitive when expense is an issue, which is generally the case for animal vaccines. For domestic livestock the level of protection produced in response to vaccination does not have to be outstanding to present a clear economic advantage for administering a relatively inexpensive vaccine. For many livestock diseases there are currently no suitable vaccine candidates due, in part, to the costs of vaccine development. In these cases, plant-based vaccine production is an alternative method of developing cost effective vaccines. Edible plant-based vaccines are particularly well suited for delivery to animals where administering injections is a very difficult and time-consuming undertaking (e.g. aquatic species).

There are several instances in which the delivery of an edible plant-based vaccine to animals can also serve to protect human health. Rabies (21) and *E. coli* O157:H7 (14) are examples of two target diseases for which plant based vaccines are in the early stages of development and the use of these vaccines would impart protection to both animals and humans. Other potential targets include *Salmonella* and West Nile virus. With regard to rabies and West Nile virus, it may ultimately be possible to vaccinate wild populations of at risk animals.

Conclusions

The technology for plant-based edible vaccines has advanced rapidly over the last fifteen years. There has been substantial progress in raising antigen expression to levels that are practical for the delivery of required oral doses. More recently, progress has been made in the development of practical delivery forms for plant-based vaccines. Early phase clinical trials and target animal trials have provided promising efficacy data with no indication of safety concerns. Due to the lower costs and shorter timelines of target animal trials compared to clinical trials, the first commercial plant-based vaccines are likely to be directed against animal diseases. There are several disease targets where strong economic arguments favour the development of edible plant-based vaccines. The forthcoming commercialisation of leading vaccine candidates will make the next decade very exciting.

Vaccins végétaux pour la santé animale

S.J. Streatfield

Résumé
Les vaccins végétaux sont des vaccins recombinants à base de sous-unités protéiques. En principe, le choix de l'espèce végétale utilisée pour produire l'antigène doit assurer l'innocuité d'une administration orale sous la forme d'un vaccin comestible. Ces vaccins sont bien adaptés à la lutte contre les maladies pour lesquelles on dispose d'un antigène candidat approprié permettant de produire le vaccin et en cas de coût de production ou de distribution prohibitif du vaccin actuel. Plusieurs groupes universitaires et industriels de recherche étudient actuellement l'utilisation des vaccins végétaux à la fois chez l'homme et chez les animaux. À ce jour, les projets relatifs aux vaccins humains les plus avancés ont achevé avec succès les études cliniques de phase I et les projets portant sur les vaccins animaux ont produit des données prometteuses dans le cadre des essais de phase initiale ciblant des espèces animales spécifiques. Le présent article présente les avantages offerts par les vaccins végétaux, indique les progrès accomplis pour les vaccins candidats les plus avancés et expose les prévisions concernant une utilisation future des vaccins végétaux. L'article est principalement consacré à l'application des vaccins végétaux dans le domaine vétérinaire, en particulier leur utilisation chez les animaux d'élevage domestiques, mais donne aussi des exemples d'utilisation de ces vaccins dans le cadre de la médicine humaine.

Mots-clés
Administration orale – Production de vaccin – Production de vaccin en vrac – Santé animale – Stockage des vaccins – Vaccin comestible – Vaccin oral – Vaccin sous-unité – Vaccin végétal – Vaccin vétérinaire.

Vacunas vegetales de uso animal

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Resumen
Las vacunas producidas a partir de plantas son preparados recombinantes a base de subunidades proteicas. Para producir un antígeno dado, es preciso elegir una especie botánica cuya administración oral, en forma de vacuna comestible, no presente riesgos. Se trata de vacunas idóneas para prevenir enfermedades contra las que se dispone del antígeno adecuado para producirlas y, también, en los casos en que el costo de producción o de distribución de la vacuna existente es prohibitivo. Actualmente, varios grupos industriales y universitarios están investigando la utilización de vacunas vegetales, tanto en seres humanos como en animales. Hasta la fecha, los proyectos más adelantados sobre vacunas para seres humanos han superado con éxito la fase I de las pruebas clínicas; además, los proyectos sobre vacunas de uso animal han dado resultados prometedores respecto a determinadas especies animales en las pruebas de la fase inicial. En este artículo se describen las ventajas de las vacunas vegetales, se indican los progresos realizados con las vacunas más avanzadas y se exponen las previsiones sobre las utilizaciones

futuras de las vacunas vegetales. Si bien el tema central de este artículo es la aplicación de vacunas vegetales en la sanidad animal y, en particular, en la cría doméstica de animales, también se examinan ejemplos de su utilización en la salud humana.

Palabras clave
Administración oral – Almacenamiento de vacunas – Producción de vacunas – Producción en masa de vacunas – Sanidad animal – Vacuna comestible – Vacuna de subunidades – Vacuna oral – Vacuna vegetal – Vacuna veterinaria.

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