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Transgenic Plant Vaccine: A Breakthrough in Immunopharmacotherapeutics

Awale MM*, Mody SK, Dudhatra GB, Avinash Kumar, Patel HB, Modi CM, Kamani DR and Chauhan BN

Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India

Abstract

Transgenic plant vaccines are genetically engineered plant vaccines in which a selected gene is encoded for the desired antigen and modified which when taken orally elicits a strong immune response in the body. Plant-cell-produced vaccines are inherently safe because they pose no risk of microbiologic contamination associated with animal-derived vaccines and eliminate the risk of pathogenicity, reversion to virulence and shedding. Oral delivery stimulates mucosal immunity (the first line of defense) in the tissues lining the respiratory system and eliminates injection-related hazards. Plants structure may help in maintaining the antigenic property even after degradation in intestine. Plenty of availability of plants makes the vaccine production of low cost apart from low cost in storage and transportation. They act through different mechanism of action mainly stimulating the lymphoid structure in the intestine. This review highlights the development of transgenic plant vaccine, its action, and certain important diseases of animals, poultry and humans and status of plant vaccine developed against them.
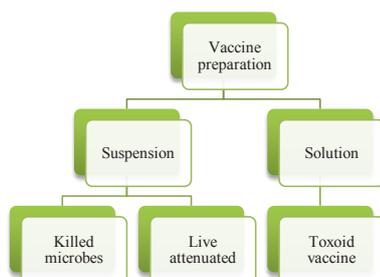
Keywords: Transgenic plant; Vaccine; Oral delivery; Immunopharmacology; Therapeutics

Introduction

Prior to vaccination, inoculation was practiced, and brought to the West in 1721 by Lady Mary Wortley Montagu, who showed it to Hans Sloane, the King's physician. Sometime during the 1770s Edward Jenner heard a milkmaid boast that she would never have the often-fatal or disfiguring disease smallpox, because she had already had cowpox, which has a very mild effect in humans. In 1796, Jenner took pus from the hand of a milkmaid with cowpox, inoculated an 8-year-old boy with it, and six weeks later variolated the boy's arm with smallpox, afterwards observing that the boy did not catch smallpox [1]. Further experimentation demonstrated the efficacy of the procedure on an infant [2]. Since vaccination with cowpox was much safer than smallpox inoculation the latter, though still widely practiced in England, was banned in 1840 [3]. Louis Pasteur generalized Jenner's idea by developing what he called a rabies vaccine, and in the nineteenth century vaccines were considered a matter of national prestige, and compulsory vaccination laws were passed [4]. Further many scientist developed a number of vaccines for a number of disease (Table 1).

Classification of vaccines

Presently vaccines that are available commercially can broadly be classified as:

Other classification

1. Live vaccines

Disease	Scientist	Source	Year
Rabies	Louis Pasteur and Emile Roux	Virus harvested from dead rabbits	1885
Polio	1.Transmitted to monkeys– Landsteiner and popper	-------	1908
	2. Preparation of serum by inoculating in horse- Pettit	-------	1917
	3. Same by using chimpanzee (neither of them proved effective than Netters serum)	-------	1932
Rotaviral diarrhea	Ruth bishop was first to describe the virus	From the faeces of children	1973
	Thomas Henry Flewett suggested the name rota virus	-------	1974
	First licensed vaccine	Cell cultures developed from monkey kidneys + trypsin	1998
Infectious bursal diarrhea	The virus recognized	-------	1967
Foot and mouth disease	Friedrich Loeffler	Described the cause and showed virus in the filterate	1897
	Waldmann and Pape	Used animal model to show the virus	

------- : Source not mentioned.

Table 1: Development of conventional vaccines with their inventor [4].

*Corresponding author: Dr. Madhavi Awale, Department of Pharmacology and Toxicology, College of Veterinary Science and Animal Husbandry, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat, India-385506, Tel: +91 8000410770; E-mail: madhavi_awale@yahoo.co.in

2. Killed vaccines

3. Bacterial cell component vaccines

4. Toxoid vaccines

5. Viral sub unit vaccines

6. Synthetic peptides

Dangers of Vaccination

Although vaccination proved to be a major milestone in the scientific development yet there still is disagreement over its use. Some of the insidious side effects of the elements and substances used in vaccine serums include: blood disorders, auto-immune diseases, cerebral palsy, brain damage, paralysis, neurological impairment, monkey fever, Gullian-Barre-syndrome; autism, mental retardation, premature aging, cancer (and leukemia); multiple sclerosis, SIDS (sudden infant death syndrome), asthma and bronchitis, malaise, convulsions (epilepsy); seizure, encephalopathy (degenerative disease of the brain); thrombocytopenia (inflammation of veins), Cochlear lesion (loss of function of the inner ear); brachial plexus neuropathies (nervous disease of the arms, nerves, and lymphatic); erythema (morbid redness of the skin); shock episodes (excessive screaming), chronic melancholy (child never smiling or laughing), learning disability. Hence there is a need to find an alternative to these present vaccines. This alternative can be substituted by development of plant vaccines.

Why do we need to use Plant Vaccines?

Plant systems do not harbor human or animal pathogens (such as virions or prions) and, therefore, they do not transmit such pathogens along with the target subunit vaccine [5]. Moreover, they cost less to produce than via fermentation or bioreactors; plants can be grown in the field or in a greenhouse relatively inexpensively. When produced in edible parts of the plant, such as grain, fruit or even leaves, subunit vaccines may not require purification. Maintaining the antigenic protein within plant cells that are edible may also contribute to stability and reduce degradation. Another advantage of producing subunit vaccines in edible parts of a plant is the potential to deliver the orally rather than intramuscularly, providing a simple and easy means of administration to humans and animals [6]. Moreover, oral delivery stimulates mucosal immunity (the first line of defense) in the tissues lining the mouth, nose and esophagus (among others) that provide the first target of opportunity for pathogens to enter and infect the human body [7]. In addition, production in plants reduces the overall cost of vaccinations, which is often prohibitive in developing countries; for example, sterile hypodermic syringes are not required [8].

General Consideration of Plant Vaccines

History

Plant vaccines can safely replace the current vaccines. The first vaccine in plants was produced by expressing the *Streptococcus mutans* surface protein antigen A (SpaA) in tobacco [9]. This was followed by expressing hepatitis B surface antigen in transgenic plants [10]. Since then, several proteins of different origin have been expressed in plants. These include *Escherichia coli* heat-labile enterotoxin antigen [11], Enkephalins [12], Human serum albumin [13], Glucocerebrosidase and granulocyte macrophage colony stimulating factor [14], Norwalk virus surface protein [15], VP1 antigen from foot and mouth disease virus [16,17], cholera toxin B subunit [18], Rabies antigen [19], the S protein of transmissible gastroenteritis coronavirus [20,21], Respiratory

synctial virus G and F proteins [8,22], the VP6 protein of rotavirus [23], the measles [24] and Rinderpest [25], virus hemagglutinin proteins and an epitope from the major surface antigen of *Plasmodium falciparum* [26].

Mechanism of action

Most pathogens enter the mucosal surface lining epithelium of digestive, respiratory and urino-reproductive tracts which are collectively the largest immunologically active tissue in the body [27]. The mucosal immune system is the first line of defense and the most effective site for vaccination against those pathogens, nasal, oral vaccine being the most effective. The goal of the vaccine is to stimulate both mucosal and humoral immunity against pathogens. Edible vaccines when taken orally undergo mastication process and the majority of the degradation occurs in the intestine as a result of action of the bacterial enzymes on edible vaccines. Peyers patches are an enriched source of IgA producing plasma cells and have the potential to populate mucosal tissue and serves as the mucosal immune effector sites. The breakdown of the edible vaccines occurs near PP, which consists of 30-40 lymphoid nodules on the outer surface of the intestine and contains follicles from which germinal centre develops upon antigenic stimulation. These follicles act as the sites from which antigen penetrates the intestinal epithelium thereby accumulating antigen within organized lymphoid structure. The antigen then comes in contact with the M cells. It contacts with the lumen with the broad membrane processes and contains the deep invagination in the basolateral plasma membrane. This pocket is filled with a cluster of B-cells, T-cells and macrophages. M cells express class II MHC molecules and antigens transported across the membrane by M cells can activate the B- cells within these lymphoid cells. The activated B-cells leave the lymphoid follicles and migrate to the diffused mucosal associated lymphoid tissue where they differentiate into plasma cells that secrete IgA class of antibodies [28]. Thus these antibodies then interact with antigen in the lumen of the intestine [6].

Vaccine formulation

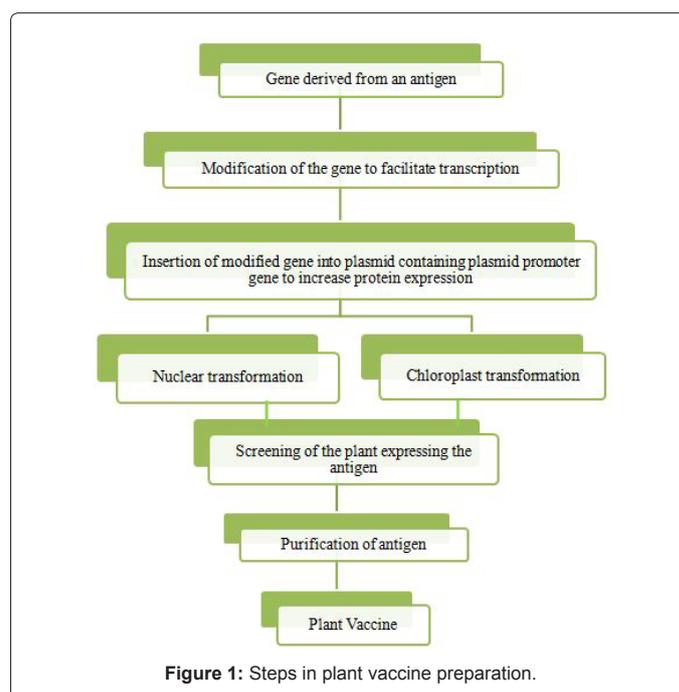General procedure of preparation of plant vaccines is given in figure 1.

Figure 1: Steps in plant vaccine preparation.

Some Important Diseases and their Vaccines

Rabies

Causative organism is *lyssa* virus belonging to family *Rhabdoviridae*. Single stranded negative sense RNA virus. It has cylindrical shape with bullet shaped virus particle.

The commonest mode of transmission in man is by the bite of a rabid animal or the contamination of scratch wounds by virus- infected saliva. However, other routes have been implicated in the past, such as through mucous membranes of the mouth, conjunctiva, anus and genitalia. Infection by aerosol transmission had been demonstrated in experimental animals and has been implicated in human infection in rabies-infected bat caverns and in several laboratory accidents. Man to man transmission by transplantation of infected corneas was reported in 5 instances. Rabies is an acute infection of the CNS which is almost invariably fatal. The virus is similar to Vesicular Stomatitis Virus of cattle. Following inoculation, the virus replicates in the striated or connective tissue at the site of inoculation and enters the peripheral nerves through the neuromuscular junction. It then spreads to the CNS in the endoneurium of the Schwann cells. Terminally, there is widespread CNS involvement but few neurons infected with the virus show structural abnormalities. The nature of the profound disorder is still not understood.

Conventional vaccines: Several types of live attenuated vaccines are available for use in animals, but they are considered to be unsuitable for humans. The vaccines which are available for humans at present are inactivated whole virus vaccines.

Nervous tissue preparation: This consisted of a 5% suspension of infected animal nervous tissue which had been inactivated (e.g. the sample vaccine was derived from phenol-inactivated infected rabbit brain). These preparations are now out of date as they were associated with the rare complication of demyelinating allergic encephalitis. This appears to be related to myelin basic protein in the vaccine. This complication was shown to occur in 4.6 cases for 1000 persons vaccinated by the Sample vaccine. The case-fatality proportion was 3.13%. The sample vaccine is still used in some developing countries. A suckling mouse brain vaccine is used in some Central and S. American countries.

Duck embryo vaccine: This vaccine strain is grown in embryonated duck eggs and is inactivated with B-propriolactone. This vaccine has a lower risk of allergic encephalitis. However, it is considerably less immunogenic and does have minor side effects. Almost all vaccines experience local reactions, 33% have constitutional symptoms such as fever, malaise, myalgia, and generalized lymphadenopathy.

Human diploid cell vaccine (HDCV): HDCV was introduced in 1978. It is a grown on WI-38 (U.S.) or MRC-5 (Europe) cells. The vaccine is highly effective, in several studies; antibodies have been demonstrated in 100% of all recipients. Serious adverse reactions to HDCV are extremely rare. However, the vaccine is very expensive ($100 for 6 doses), as human cell cultures are more difficult to handle than other animal cell culture systems. 5 or 6 doses of the vaccine are normally i.m. However, several studies suggest than smaller i.d. doses of HDCV may be as effective and thus it may be considered for use in poor developing countries.

Plant vaccine: In case of rabies, stable expression of the rabies surface protein was noticed in transgenic tomato but immunoprotective ability was not reported [19]. A synthetic gene coding for the surface glycoprotein (G-protein) of rabies virus identified as the major antigen that induces protective immunity was strategically designed to achieve high level expression in transgenic plants [29]. Glycosylation of the G-protein is required for immunoprotection by the rabies vaccines [30]. The native signal peptide was replaced by that of the pathogenesis related protein, PR-S of *Nicotiana tabacum*. An endoplasmic reticulum retention signal was included at C-terminus of the G-protein [13]. Tobacco plants were genetically engineered by nuclear transformation. Selected transgenic lines expressed the chimeric G-protein at 0.38% of the total soluble leaf protein. Mice immunized intraperitoneally with the G-protein purified from tobacco leaf microsomal fraction elicited high level of immune response as compared to the inactivated commercial viral vaccine. The plant-derived G-protein induced complete protective immunity in mice against intracerebral lethal challenge with live rabies virus. The result established that plants can provide a safe and effective production system for the expression of immunoprotective rabies virus surface protein [4].

Foot and mouth disease

The foot-and-mouth disease virus is the pathogen that causes foot-and-mouth disease. It is a picornavirus, the prototypical member of the *Aphthovirus* genus. The main seven serotypes are O, A, C, SAT-1, SAT-2, SAT-3, and Asia-1. The disease, which causes blisters in the mouth and feet of bovids and other cloven-hoofed animals, is highly infectious and a major plague of animal farming [31].

The virus particle (25-30 nm) has an icosahedral capsid made of protein, without envelope, containing a single strand of ribonucleic acid (RNA) containing a positive encoding of its genome. Transmission can be spread by infected animals through aerosols, through contact with contaminated farming equipment, vehicles, clothing or feed, and by domestic and wild predators.

In 1898, foot-and-mouth disease (FMD) earned a place in history as the first disease of animals shown to be caused by a virus. The three basic phases of FMD pathogenesis *in vivo* will be dissected and characterized as: (i) pre-viraemia characterized by infection and replication at the primary replication site(s), (ii) sustained viraemia with generalization and vesiculation at secondary infection sites and (iii) post-viraemia/convalescence including resolution of clinical disease that may result in long-term persistent infection.

Conventional vaccines: Killed (inactivated) vaccines against FMDV are produced by growing virus in cell culture, inactivating the virus and combining it with an adjuvant, a substance which enhances the immune response. Further processing may be carried out to concentrate the antigen to reduce the volume required for vaccination of each animal, and allow storage of antigen for prolonged periods without loss of efficacy.

Plant vaccine: It has been previously reported that the FMDV structural protein VP1 (Viral Protein), which carries critical epitopes responsible for the induction of protective neutralizing antibodies, could be successfully expressed as an immunogenic antigen in *Arabidopsis thaliana* alfalfa and potato and used, as experimental immunogen, for eliciting a virus-specific protective antibody response when parenterally or orally administered [32]. The development of a methodology based in the construction of a fusion protein composed of a very well known and easily detectable reporter gene, glucuronidase (*gus* A), fused to an epitope of interest, the antigenic determinants comprised by amino acid residues 135–160 from the structural protein VP1 of FMDV (VP135–160). The results obtained demonstrated

that a large number of individuals can be readily screened by their ß-glucuronidase (ßGUS) enzymatic activity which correlates with the levels of VP135–160 expression. Mice immunized using the selected plants readily developed a strong and protective antibody response against virulent FMDV in experimental hosts [32,33].

Status of the plant vaccine: Nevertheless, in all those cases, the concentration of the expressed protein in the transgenic plant tissues was relatively poor. Both, the difficulty in detecting the foreign protein in the plant extract by Western blot, as well as the necessity of numerous immunizations in order to induce a significant immune response indicate the low level of the expressed protein.

Thus, although the expression of immunogenic antigens in transgenic plants appears to be a very promising alternative to other methodologies for the production of recombinant proteins, its main disadvantage consists of the low concentration reached by the foreign protein in the plant tissues. This point became particularly relevant in those cases where the plant extracts are expected to be used without any further processing. Thus, increase in the concentration of the foreign protein in the transgenic plants becomes a critical issue to be considered. Among other strategies, which included genetic manipulations, an alternative to solve this problem could be the feasibility of identifying those transgenic individuals expressing exceptionally high levels of the recombinant protein.

Newcastle disease

Newcastle disease is an acute viral disease of domestic poultry and many other bird species. It is a worldwide problem that presents primarily as a respiratory disease, but depression, nervous manifestations, or diarrhea may be the predominant clinical form. Mortality is variable.

Newcastle disease is caused by an RNA virus, Newcastle disease virus (NDV), synonymous with avian paramyxovirus-1 which is in the genus Avulavirus, family *Paramyxoviridae*. Isolates are classified into 1 of 3 virulence groups by chicken embryo and chicken inoculation as virulent (velogenic), moderately virulent (mesogenic), or of low virulence (lentogenic). Lentogenic strains are used widely as live vaccines in healthy chickens. Clinical manifestations vary from high morbidity and mortality to asymptomatic infections.

Virulent NDV strains are endemic in poultry in most of Asia, Africa, and some countries of North, Central, and South America. Other countries, including the USA and Canada, are free of those strains and maintain that status with import restrictions and eradication by destroying diseased poultry. Cormorants, pigeons, and imported *psittacine* species have also been sources of virulent NDV infections of poultry. Low virulence NDV is prevalent in poultry and wild birds, especially waterfowl. Infected birds shed virus in exhaled air, respiratory discharges, and faeces. Virus is shed during incubation, during the clinical stage, and for a varying but limited period during convalescence. Virus may also be present in eggs laid during clinical disease and in all parts of the carcass during acute virulent infections. Chickens are readily infected by aerosols and by ingesting contaminated water or food. Infected chickens are the primary source of virus, but other domestic and wild birds may be sources of NDV. Transfer of virus, especially in infective faeces, by the movement of people and contaminated equipment is the main method of spread between poultry flocks.

Conventional vaccines

There are two types of vaccines used:

Strain	Description
F	Lentogenic. Usually used in young chickens but suitable for use as a vaccine in chickens of all ages.
B1	Lentogenic. Slightly more virulent than F, used as a vaccine in chickens of all ages.
La Sota	Lentogenic. Often causes post vaccination respiratory signs, used as a booster vaccine in flocks vaccinated with F or B1.
V4	Avirulent. Used in chickens of all ages.
V4-HR	Avirulent. Heat Resistant V4, thermostable, used in chickens of all ages.
I-2	Avirulent. Thermostable, used in chickens of all ages.
Mukteswar	Mesogenic. An invasive strain, used as a booster vaccine. Can cause adverse reactions (respiratory distress, loss of weight or drop in egg production and even death) if used in partially immune chickens. Usually administered by injection.
Komarov	Mesogenic. Less pathogenic than Mukteswar, used as booster vaccine. Usually administered by injection.

Table 2: Strains of Newcastle disease used in live vaccine.

Killed vaccines: The ability of the virus to infect cells has been destroyed by treatment with a chemical, radiation or heat. These vaccines invoke only a circulating antibody response.

Live vaccines: These vaccines are made with virus that is alive and able to infect cells. Strains of virus of low or moderate virulence are used. They mimic natural infection and induce all three immune responses. There are total eight strains of Newcastle disease virus used in live vaccines (Table 2).

Thermostable Newcastle disease vaccines: Thermostable Newcastle disease vaccines exhibit a relative resistance to inactivation on exposure to elevated temperatures. Strains of Newcastle disease virus vary in thermostability. Thermostable vaccines are prepared from a strain of Newcastle disease virus that retains its ability to infect cells after storage outside a cold chain for a short period of time.

Plant vaccine: Transgenic plant has become an attractive bioreactor to produce high-value medical peptides and proteins in biomedical research. In present study, two expression cassettes, pUNDVF and pGNDVF containing the fusion protein gene of Newcastle disease virus (NDV-F) under the control of maize ubiquitin (Ubi) promoter or rice glutelin (Gt1) promoter, respectively, were constructed, and introduced into rice (*Oryzy sativa* L.) by *Agrobacterium*-mediated transformation. A total of 12 independent transgenic rice lines were regenerated, and the result from PCR analysis indicated that the T-DNA region containing the NDV-F chimeric gene had been integrated into the genome of transgenic rice plants. ELISA and Western-blot analyses revealed that the NDV-F protein could be expressed and accumulated in both leaf and seed tissue of several transgenic rice plants. Moreover, the immunogenicity of expressed proteins was tested in a mouse model and the results showed that specific antibodies were elicited in mice immunized intraperitoneally with crude protein extracts from transgenic rice plants. It implied the potential of using transgenic rice-based expression systems as supplementary bioreactor for NDV engineering subunit vaccine [34].

Infectious bursal disease

Infectious bursal disease (IBD) is a highly contagious disease of young chickens caused by infectious bursal disease virus (IBDV) [35], characterized by immunosuppression and mortality generally at 3 to 6 weeks of age. The disease was first discovered in Gumboro, Delaware in 1962. BDV is a double stranded RNA virus that has a bi-segmented genome [35] and belongs to the genus *Avibirnavirus* of family *Birnaviridae*. There are two distinct serotypes of the virus, but

only serotype 1 viruses cause disease in poultry. The virus is attracted to lymphoid cells and especially those of B-lymphocyte origins. Young birds at around two to eight weeks of age that have highly active bursa of Fabricius are more succeptible to disease. Birds over eight weeks are resistant to challenge and will not show clinical signs unless infected by highy virulent strains.

Under natural conditions, the most common mode of infection appears to be via the oral route. From the gut, the virus is transported to other tissues by phagocytic cells, most likely resident macrophages. Although viral antigen has been detected in liver and kidney within the first few hours of infection, extensive viral replication takes place primarily in the bursa of Fabricius.

After ingestion, the virus destroys the lymphoid follicles in the Bursa of Fabricius as well as the circulating B-cells in the secondary lymphoid tissues such as GALT (gut-associated lymphoid tissue), CALT (conjunctiva), BALT (Bronchial) caecal tonsils, Harderian gland, etc. Acute disease and death is due to the necrotizing effect of these viruses on the host tissue. If the bird survives and recovers from these phases of the disease, they remain immunocompromised which means they are more susceptible to other diseases and vaccination in the face of outbreak will not be effective.

The acute lytic phase of the virus is associated with a reduction in circulating IgM^+ cells although there is no detectable reduction in circulating immunoglobulins (Igs).

T cells are resistant to infection with IBDV. Although the thymus undergoes marked atrophy and extensive apoptosis of thymocytes during the acute phase of virus infection, there is no evidence that the virus actually replicates in thymic cells. Gross and microscopic lesions in the thymus are quickly overcome and the thymus returns to its normal state within a few days of virus infection.

Conventional vaccines

Live vaccines: Attenuated strains of IBD viruses are used. These are referred to as either mild, intermediate, or 'intermediate plus' ('hot') vaccines. The mild vaccines cause limited bursal damage, while the intermediate and intermediate plus vaccines cause some lymphocytic depletion in the bursa of Fabricius. Usually none of the vaccine types causes immunosuppression when used in birds over 14 days old that have been hatched from IBD immune parents [36].

Oil based vaccines: These are usually used to stimulate high and uniform levels of antibody in parent chickens so that the progeny will have high and uniform levels of MDA. The killed vaccines may occasionally be used in young valuable birds with MDA. The killed vaccines are manufactured in oil emulsion adjuvant and given by injection. They must be used in birds already sensitized by primary exposure, either to live vaccine or to field virus. This can be checked serologically. High levels of MDA can be obtained in breeder birds by giving, for example, live vaccine at approximately 8 weeks of age, followed by inactivated vaccine at approximately 18 weeks of age [37].

Immune complex vaccine: This immune complex vaccine is developed by mixing live intermediate plus infectious bursal disease virus (IBDV) with hyperimmune IBDV chicken serum (IBDV-Icx vaccine) [38].

Plant vaccine: The VP2 coding sequence was isolated and integrated into *A. thaliana* genome by *Agrobacterium tumefaciens*–mediated transformation. Soluble VP2 expressed in transgenic plants was used to immunize chickens. Chickens receiving oral immunization with plant-derived VP2 at 1 and 3 wk of age had an antibody response using enzyme-linked immunosorbent assay and 80% protection against challenge infection at 4 wk. Chickens primed with a commercial vaccine at 1 wk followed by an oral booster with VP2 expressed in plants at 3 wk of age showed 90% protection. Chickens immunized with a commercial vaccine at 1 and 3 wk showed 78% protection. Results supported the efficacy of plant-produced VP2 as a vaccine against IBD.

Rota viral disease

Rotavirus is the most common cause of severe diarrhea among infants and young children [39], and is one of several viruses that cause infections often called stomach flu, despite having no relation to influenza. It is a genus of double-stranded RNA virus in the family *Reoviridae*. By the age of five, nearly every child in the world has been infected with rotavirus at least once [40]. However, with each infection, immunity develops, and subsequent infections are less severe [41] adults are rarely affected [42]. There are five species of this virus, referred to as A, B, C, D, and E. Rotavirus A, the most common, causes more than 90% of infections in humans.

The virus is transmitted by the fecal-oral route [43]. It infects and damages the cells that line the small intestine and causes gastroenteritis. Although rotavirus was discovered in 1973 and accounts for up to 50% of hospitalizations for severe diarrhea in infants and children, its importance is still not widely known within the public health community, particularly in developing countries. In addition to its impact on human health, rotavirus also infects animals, and is a pathogen of livestock.

The diarrhea is caused by multiple activities of the virus. Malabsorption occurs because of the destruction of gut cells called enterocytes. The toxic rotavirus protein NSP4 induces age and calcium ion-dependent chloride secretion, disrupts SGLT1 transporter-mediated reabsorption of water, apparently reduces activity of brush-border membrane disaccharidases, and possibly activates the calcium ion-dependent secretory reflexes of the enteric nervous system. Ball Healthy enterocytes secrete lactase into the small intestine; milk intolerance due to lactase deficiency is a particular symptom of rotavirus infection, which can persist for weeks. A recurrence of mild diarrhea often follows the reintroduction of milk into the child's diet, due to bacterial fermentation of the disaccharide lactose in the gut [44].

Conventional vaccines: In 1998, a rotavirus vaccine was licensed for use in the United States. Clinical trials in the United States, Finland, and Venezuela had found it to be 80 to 100% effective at preventing severe diarrhea caused by rotavirus A, and researchers had detected no statistically significant serious adverse effects. The manufacturer, however, withdrew it from the market in 1999, after it was discovered that the vaccine may have contributed to an increased risk for intussusception, a type of bowel obstruction, in one of every 12,000 vaccinated infants. The experience provoked intense debate about the relative risks and benefits of a rotavirus vaccine. In 2006, two new vaccines against rotavirus A infection were shown to be safe and effective in children, and in June 2009 the World Health Organization recommended that rotavirus vaccination be included in all national immunization programmes to provide protection against this virus.

Plant vaccine: A critical factor in edible plant-derived vaccine development is adequate expression of the exogenous antigens in transgenic plants. A codon-optimized gene (sVP6) encoding the VP6 protein of human group A rotavirus was synthesized and inserted it into the alfalfa genome using *Agrobacterium*-mediated transformation.

As much as 0.28% of the total soluble protein of the pBsVP6-transgenic alfalfa was sVP6. Female BALB/c mice were gavaged weekly with 10 mg of transgenic alfalfa extract containing 24 µg of sVP6 protein and 10 µg of CpG-rich oligodeoxynucleotides as mucosal adjuvant. Immunized mice developed high titers of anti-VP6 serum IgG and mucosal IgA. Offspring of immunized dams developed less severe diarrhea after challenge with simian rotavirus SA-11, indicating that antibodies generated in the dams provided passive heterotypic protection to the pups. These results suggest that oral immunization with pBsVP6-transgenic alfalfa provides a potential means of protecting children and young animals from severe acute rotavirus-induced diarrhea [45].

Hepatitis

Hepatitis B is an infectious illness caused by hepatitis B virus (HBV) which infects the liver of hominoidea, including humans, and causes an inflammation called serum hepatitis [46]. Originally known as "serum hepatitis", the disease has caused epidemics in parts of Asia and Africa, and it is endemic in China [47]. About a third of the world's population, more than 2 billion people have been infected with the HBV. HBV is a hepadnavirus - *hepa* from hepatotrophic and DNA because it is a DNA virus and it has a circular genome composed of partially double-stranded DNA. The viruses replicate through an RNA intermediate form by reverse transcription, and in this respect they are similar to retroviruses. Although replication takes place in the liver, the virus spreads to the blood where virus-specific proteins and their corresponding antibodies are found in infected people.

Transmission of HBV results from exposure to infectious blood or body fluids containing blood. Possible forms of transmission include sexual contact, blood transfusions, re-use of contaminated needles and syringes, and vertical transmission from mother to child during childbirth. Without intervention, a mother who is positive for HBsAg confers a 20% risk of passing the infection to her offspring at the time of birth. This risk is as high as 90% if the mother is also positive for HBeAg. HBV can be transmitted between family members within households, possibly by contact of non intact skin or mucous membrane with secretions or saliva containing HBV.

HBV primarily interferes with the functions of the liver by replicating in liver cells, known as hepatocytes. The receptor is not yet known, though there is evidence that the receptor in the closely related duck hepatitis B virus is carboxypeptidase D [48]. HBV virions (DANE particle) bind to the host cell via the preS domain of the viral surface antigen and are subsequently internalized by endocytosis. PreS and IgA receptors are accused of this interaction. HBV-preS specific receptors are primarily expressed on hepatocytes; however, viral DNA and proteins have also been detected in extrahepatic sites, suggesting that cellular receptors for HBV may also exist on extrahepatic cells.

During HBV infection, the host immune response causes both hepatocellular damage and viral clearance. Although the innate immune response does not play a significant role in these processes, the adaptive immune response, particularly virus-specific cytotoxic T lymphocytes (CTLs), contributes to most of the liver injury associated with HBV infection. CTLs eliminate HBV infection by killing infected cells and producing antiviral cytokines, which are then used to purge HBV from viable hepatocytes. Although liver damage is initiated and mediated by the CTLs, antigen-nonspecific inflammatory cells can worsen CTL-induced immunopathology, and platelets activated at the site of infection may facilitate the accumulation of CTLs in the liver.

Plant Vaccine: Tobacco plants were genetically transformed with the gene encoding hepatitis B surface antigen (HBsAg) linked to a nominally constitutive promoter. Enzyme-linked immunoassays using a monoclonal antibody directed against human serum-derived HBsAg revealed the presence of HBsAg in extracts of transformed leaves at levels that correlated with mRNA abundance. This suggests that there were no major inherent limitations of transcription or translation of this foreign gene in plants. Recombinant HBsAg was purified from transgenic plants by immunoaffinity chromatography and examined by electron microscopy. Spherical particles with an average diameter of 22 nm were observed in negatively stained preparations. Sedimentation of transgenic plant extracts in sucrose and cesium chloride density gradients showed that the recombinant HBsAg and human serum-derived HBsAg had similar physical properties. Because the HBsAg produced in transgenic plants is antigenically and physically similar to the HBsAg particles derived from human serum and recombinant yeast, which are used as vaccines, we conclude that transgenic plants hold promise as low-cost vaccine production systems [49].

Limitations of plant vaccines

There may be development of immunotolerance to vaccine peptide or protein. Consistency of dosage form differs to fruit, plant, and generation of the plant. Stability of vaccine in fruit is not known. Evaluating dosage requirement is tedious in case of plant vaccine. Selection of best plant is difficult. Certain plants like potato cannot be eaten raw and cooking may change the properties of vaccine [6,50].

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