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PLANTIBODY: AN OVERVIEW

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Abstract

In this text we deal with the formation of plantibody or plant antibody. As plants are the major sources of earth and can produce antibodies which can be used for human use without any interaction of any types so we explore plant proteins for formation of antibodies for human purpose. These plantibodies are formed by various methods like conventional method, cell tissue culture method, breeding and sexual crossing, transgenic seeds, targeting and compartmentalizing. These are further purified by various methods like filtration, chromatography, diafiltration, immunofluorescence, polymer fusion and further evaluated by RIA (Radioimmunoassay), ELISA (Enzyme linked immunosorbant assay), immunofluorescence, southern blot analysis, western blot analysis, northern blot analysis. We have also cited various applications of plant antibody here.

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Introduction

A **plantibody** is an antibody produced by genetically modified crops. Antibodies are part of animal immune systems, and are produced in plants by transforming them with antibody genes from animals. Although plants do not naturally make antibodies, plantibodies have been shown to function in the same way as normal antibodies.

The use of transgenic plants for the expression of molecules with therapeutic, diagnostic or veterinary applications has been very well documented during the last 10 years. This technology represents a great opportunity for the pharmaceutical industry, since biological products now account for a large percentage of all pharmaceutical compounds. Several plant-produced antibodies (plantibodies) are undergoing clinical trials. The term "plantibodies" was created to describe the products of plants that have been genetically engineered to express antibodies and antibody fragments in plant, with many applications. Plants are being used in this technology as antibody factories, using their endomembrane and secretory systems to produce large amounts of clinically viable proteins, which can later be purified from the plant tissue. Antibodies can be expressed in plants as either full-length molecules or as smaller fragments.

Recombinant protein production using transgenic plants as bioreactors is likely to be more economical than alternative systems, especially for large-scale needs [1]. There are several advantages of the use of plants for antibody production over conventional methods, such as, potential for large-scale, low-cost biomass production using agriculture, low risk of product contamination by mammalian viruses, blood-borne pathogens, oncogenes and bacterial toxins. The capacity of plant cells to correctly fold and assemble not only antibody fragments and single chain peptides, but also full-length multimeric proteins, low downstream processing requirements for proteins administered orally, elimination of the purification requirement when the plant containing the recombinant proteins is edible, such as potatoes, the ability to introduce new or multiple transgenes by sexual crossing of plants, the avoidance of ethical problems associated with transgenic animals, formulated in seeds, plant-made enzymes have been found to be an extremely convenient method for reducing storage and shipping costs, for an indefinite amount of time, under ambient conditions, production size is flexible and easily adjustable to the needs of changing markets. Plants are also capable of synthesizing and assembling virtually any kind of antibody molecule, ranging from the smallest antigen-binding domains and fragmentsto full length and even multimeric antibodies.

Six plant-derived antibodies have been developed as human therapeutics, two of which have reached phase II clinical trials. One of these is a full-length IgG specific for EpCAM (a marker of colorectal cancer) developed as the drug Avicidin by NeoRx and Monsanto. The five remaining antibodies are CaroRx, scFvT84.66, Anti-HSV, 38C13 and PIPP (anti-hCG) [2].

Plant cells efficiently assemble multiple subunit proteins such as mAbs and perform necessary posttranslational modifications on transgenic proteins.

Methods for Plantibody production

One of the several methods for synthesizing plantibody is conventional method which uses transformation and transient expression to

introduce new genes into a host cell. The transformant cell is then introduced into the plant embryo, propagation of plant in open field allow large-scale production of antibodies.

Plant tissue culture is the most economic and time saving method for production of antibodies from plants. In this plant cells in differentiated states are grown in bioreactors with foreign proteins harvested from either the biomass or culture liquid. Cell cultures contain fewer biological proteins or molecules (along with herbicides and pesticides) than open field plants or bacterial/yeast cell cultures, which may contaminate the product [3].

An experiment on tobacco plant established is breeding and sexual crossing as a method for production of plantibody. In this experiment, transformation was used to introduce kappa-chains of either light or heavy regions into tobacco plants. The same was done with gamma-chains of either light or heavy regions. Upon crossing one plant with kappa-chains and another plant with gamma-chains, an antibody was produced that expressed both chains [4-5].

Some researches suggest use of transgenic seeds in place of green plant tissue as plants cannot store antibodies for an extended period of time. Seeds contain a low level of proteases that allows proteins to be stored without degradation [6-7].

Antibodies can be targeted to some compartments by the tagging with a small peptide sequence. This allows antibodies to be protected from proteases that exist in the cytoplasm of cells [8].

Purification techniques for Plantibody

Easy purification of plantibody makes biopharmaceutical production economic [9]. Transgenic seeds assure excellent storage properties and thus added flexibility in processing management and batch production. Because of limited range of endogenous proteins in seeds separation of plantibody is less complicated [8].

Absence of human pathogens in plants eliminates expensive validation of virus-removal steps during purification. However, the probability of presence of a diverse bioburden on or in plants grown outdoors in non-sterile conditions is more. So, processes for elimination or minimization of contamination with endotoxins and mycotoxins will be necessary in every commercial process to purify antibodies. Phenolics can interact with proteins in ways that can irreversibly alter the properties of proteins [10]. Most of the phenolics released during extraction are small in size, water soluble, and removable by ultrafiltration/ diafiltration steps.

Techniques for Purification of Plantibody

- Filtration
- Immunofluoresence
- Chromatography
- Diafiltration
- Polymer fusion
- The 'plantibody' (functional IgG antibody produced in plants) was easily purified by Protein A-Sepharose chromatography with a yield of approximately 35 µg/g of fresh leaf material, and its glycosylation indicated that, irrespective of the KDEL signal, the molecule is modified in both the ER and Golgi.

Evaluation techniques for the Plantibody

- RIA(Radioimmunoassay) Northern blot analysis
- ELISA (Enzyme linked immuno sorbent assay) Western blot analysis
- Immunofluorescence Southern blot analysis

Applications of plant antibody

Therapeutic applications

Therapeutic applications of plantibody are the treatment of infectious disease, inflammation, autoimmune disease or cancer. Tobacco produced mAb is more viable alternative to mAb produced in mouse ascites fluid for the large amounts needed for purification of hepatitis B vaccine. Plant produced antibodies have also been investigated for inflammatory disease and to induce tolerance.

Plant produced the world's first clinically tested Plantibody, CaroRx. CaroRx binds specifically to *Streptococcus mutans*, the bacteria that cause tooth decay, and prevents the bacteria from adhering to teeth. CaroRx is intended for regular topical preventative administration by both dental hygienists and patients allowing a thorough cleaning and intervention for any existing decay.

Immunization

The production of proteins in plants is attractive task for producing pharmaceutical polypeptides. Potential proteins produced are cytokines, hormones, enzymes, epidermal growth factors, interferons, human protein C, and pharmaceutical foodstuff which are considered for oral immunization [11]. Plants produce different classes of proteins which are inexpensive and have increased pharmaceutical value. Due to all these reasons transgenic plants are better alternative. Oral vaccines offer convenient immunization strategies for implementing universal vaccination programs throughout the world [12].

The pathogen generally attack human host at mucosal sites in the respiratory tract, gastrointestinal tract or genital tract so stimulation of immune responses of suitable strength and quality to protect against illness is particularly desirable at these sites. This can be achieved by applying the vaccine directly to the mucosal surface, inducing systemic and cellular immune responses as well as local immune responses. Mucosal vaccines produce stronger immune responses directed at the initial site of interaction between the pathogen and host. Infectious agents colonizing epithelial membranes include bacteria and viruses transmitted in contaminated food, water or by sexual contact. Transgenic plants expressing antigens are used as an inexpensive oral-vaccine production and delivery system [8] so immunization is possible through consumption of an "edible vaccine" to provide passive immunization. Genetically engineered plants and plant viruses are also used to produce vaccines against several human diseases for life-threatening infections such as diphtheria, cholera and AIDS [3]. Some of the proteins are potent inducers of immune responses but some immunizing proteins may not work well when taken orally. Oral vaccines must be protected during passage through the hostile environment of the stomach and intestine to the sites of immune stimulation. Variety of delivery systems is developed for presenting nonliving antigens to mucosal surfaces allowing these antigens to persist and survive in the hostile gastric and enteric environments. These include polylactide/polyglycolide, microspheres, liposomes, proteosomes, cochleates, virus-like particles, and immune-stimulating complexes [13-16].

Immunization at mucosal surfaces and development of SIgA response are the basis of the success of oral vaccines against polio, rubella virus, adenoviruses, influenza A, rotavirus, salmonella, and cholera [17]. Passive transfer of maternal SIgA through milk is the

mechanism in mammals that prevents mucosal infection in newborns lacking a fully matured immune system. Immunoglobulin A (IgA) is the most suitable class for oral administration, however, production of monoclonal antibodies (mAbs) of IgA class has been challenging. We succeeded in production of IgA mAbs by means of intranasal immunization and hybridoma production using nasal-associated lymphoid tissues from mice. IgA class plantibodies may be served as a high profile functional food such as salad. It will contain edible vegetables, such as lettuce and cabbage containing plantibodies, together with dressing that contains IgA antibodies in a form of emulsion.

Transgenic potato

Successful outcomes of studies involving CTB and LT-B in mice led to experimentation with human subjects hypothesized that mucosal immune response would occur upon ingestion of raw potato tubers expressing CTB or LT-B. The results show that vaccines from plants can be taken orally through food substances. As raw potato cannot be taken as such so transgenic potatoes developed which could be boiled for 3 minutes with only a 50% loss of LT-B.

Prevention and Treatment of Bacterial Infections

Infectious diseases are a significant cause of morbidity and mortality worldwide, accounting for approximately 50% of all deaths in tropical countries and as much as 20% of deaths in the America [18]. Due to these global initiative for the development of new strategies for the prevention and treatment of infectious disease is required. From ancient times medicinal plants are clinically proven drugs, and are now re-assessed as antimicrobial agents.

Secretory IgA Antibodies

It is composed of two monomeric IgA antibody units, a small J-chain and a secretory component. SA I/II is a cell surface protein of Streptococcus mutans which can cause dental caries in humans. Secretory IgA produced by plants are similar to secretory IgA produced by animals. So, these can be used in mammals also. Oral topical treatment of this kind with plant-made SIgA appeared to be completely safe. No adverse events were reported in either trial or no signs of local or systemic side effects or hypersensitivity reactions were noted after any of the interventions or at any of the follow-up visits. No human anti-mouse antibody (HAMA) response was detected in post treatment serum samples.

Secretory IgA (SIgA) is the antibody type produced in mammals and birds protects the body from infection at mucosal surfaces. Monoclonal IgG antibodies against tumor antigens received great deal of attention scientifically and commercially as immunotherapeutic agents SIgA antibodies has only recently exploited as SIgA production *in vivo* normally requires the cooperation of two different cell types, and single animal cell systems for monoclonal SIgA production are inefficient. Transgenic plants are currently the most productive and economical system for making SIgA. Monoclonal SIgA to be tested therapeutically in a human clinical trial is a product called CaroRx, made in transgenic tobacco is designed to block adherence to teeth of the bacteria that causes cavities. This antibody accumulates to high levels in the leaves of tobacco, where it is located primarily in the endoplasmic reticulum can be efficiently purified using the affinity reagent protein G. Topical oral treatment in human subjects was safe and effective [19]. Characterization of the

expression, secretion, purification and therapeutic use of this antibody serves as a model for additional plant-made therapeutic SIgA antibodies under development.

Human Therapeutic Proteins

Plastid transformation technology is important in production of human therapeutic proteins. Protein expression achieved in plant plastids are hundreds times greater than the expression levels obtained *via* nuclear transformation. Plastids produce human proteins which are biologically active. Protein purification strategies achieving inducible plastid gene expression are developed within the system [20]. Plastid transformation technology extended to edible plant species decreases processing costs and raises the possibility of "edible protein therapies". The only limitation is that plastid-produced proteins are not glycosylated and so difficult to predict protein stability within the plastid. The high level of protein expression that can be obtained in plastids could make it possible to produce high-value therapeutic proteins in plants on a scale that could be accommodated in contained glasshouse facilities and still be economically viable.

Immunomodulation

Applications relying on modulating antigen levels *in vivo* are dependent on expression and accumulation of antibodies in specific subcellular compartments and specific tissues. Passive immunization of plants reduces infection and symptoms caused by viruses and mollicutes, and significant progress has been made towards engineering resistance against insects [21, 22]. Immunomodulation is a powerful tool for studying or altering the function of an antigen *in vivo*. Antigen, which may be an enzyme or metabolite, can either be stabilized or blocked in its action. Physiological and morphological changes were observed in plants when an artificial abscisic acid (ABA) sink was created by the production of an ABA-specific scFv in the ER of tobacco and potato plants [23-25]. Antibodies produced in plant-based expression systems are high-value products for pharmaceutical use. Plants represent cost-effective systems for the large-scale production of pharmaceuticals. For complex molecular forms sIgA, plants offer the commercially viable system for large-scale production [26]. Agro infiltration of tobacco was used to produce a diabody against carcinoembryonic antigen (CEA). Other than applications in human healthcare, plantibodies may prove useful as feed additives or for phytoremediation.

Transgenic plants are suitable for mAb production because they can be rapidly expanded in commercial production without the high-capital investment associated with traditional mAb bioreactor facilities.

Conclusion

The advancements made with transgenic plants have and will continue to have a great impact on the lives of many. Transgenic plants offer a new approach to producing and administering human antibodies. The recent research with transgenic plants has played a captivating role in providing edible vaccines, which are cheap and easy to administer. The progression of transgenic plant technology now has allowed for the progression of human life and other medicinal advancements. Hiatt was the first to demonstrate that plants could produce human antibodies and since that time, researchers have continued to build upon his findings. Since 1983, progress in the field of antibody production in plants has drastically increased, and it is projected that in

the near future, many of the necessary human antibodies will have an origin as a plantibody.

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