Plantibodies in human and animal health: a review.

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Abstract

Background: Antibodies are essential part of vertebrates' adaptive immune system; they can now be produced by transforming plants with antibody-coding genes from mammals/humans. Although plants do not naturally make antibodies, the plant-derived antibodies (plantibodies) have been shown to function in the same way as mammalian antibodies.

Methods: PubMed and Google search engines were used to download relevant publications on plantibodies in medical and veterinary fields; the papers were reviewed and findings qualitatively described.

Results: The process of bioproduction of plantibodies offers several advantages over the conventional method of antibody production in mammalian cells with the cost of antibody production in plants being substantially lesser. Contrary to what is possible with animal-derived antibodies, the process of making plantibodies almost exclusively precludes transfer of pathogens to the end product. Additionally, plants not only produce a relatively high yield of antibodies in a comparatively faster time, they also serve as cost-effective bioreactors to produce antibodies of diverse specificities.

Conclusion: Plantibodies are safe, cost-effective and offer more advantages over animal-derived antibodies. Methods of producing them are described with a view to inspiring African scientists on the need to embrace and harness this rapidly evolving biotechnology in solving human and animal health challenges on the continent where the climate supports growth of diverse plants.

Keywords: Plantibodies, plants, antibody production, bioreactors, human and animal health.

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Introduction

Antibodies, also called immunoglobulins, are a group of complex glycoproteins produced by B-lymphocytes and present in the serum and tissue fluids of vertebrates. They constitute the humoral arm of the adaptive immune system, and specifically recognize and bind to target antigens on pathogens, or toxins produced by such pathogens. This individual and specific binding activity allows antibodies to be used for a variety of applications, including the diagnosis, prevention and treatment of disease[1-4]. When a pathogen enters a vertebrate host, antibodies that can bind to antigens on the foreign agent are rapidly elicited from B-lymphocytes to combat and eliminate the pathogen. On the antibody, there is a binding region which varies depending on the conformation of the antigenic determinant (i.e. epitope) on the antigen to which it will bind, and a constant region (located on the heavy chain) which determines the class and subclass of antibody. For the binding region of antibody (i.e. the paratope) to combine with the corresponding epitope on an antigen, there must be suitable atomic groupings on opposing parts of the antigen and antibody, and the shape of the paratope must fit the epitope, so that several non-covalent bonds can form simultaneously[4].

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Defective antibody responses which result in increased susceptibility to pyogenic infections could occur due to failure of B-cell function as is seen in X-linked agammaglobulinemia, or from failure of proper T-cell signals to B-cells such as occurs in hyper-IgM syndrome, common variable immunodeficiency and transient hypogammaglobulinemia of infancy[4]. In overcoming these defects, it was discovered that the constant region of a human antibody could be transgenically combined with the binding region of an animal host, such as a mouse, to create a

recombinant antibody[5]. This antibody could then be extracted, purified and administered to combat infections in different hosts. Thus, with the advent of recombinant antibody technology, hope of an affordable treatment for diseases such as cancer and diabetes was kindled. However, although scientists could produce the antibodies, the process turned out to be very expensive and dreams of a cheap cure for fatal diseases seemed to disappear[6]. More importantly, purifying the antibodies was problematic as different chemicals affected the antibody's efficacy. Additionally, mammalian antibodies could have pathogens deleterious to humans associated with them[7] or they could be recognized by the human immune system as foreign and trigger a rejection response[8]. As a result, it was soon believed that animal antibodies were not the best option for treating diseases. For instance, despite the potential of Hu-E16, a humanized murine monoclonal antibody (mAb), and other mAbs as prophylactics and therapeutics for West Nile virus or other infectious diseases, their application may be limited by the high production costs and scalability associated with mammalian-cell culture production system. Moreover, if biological drugs are too costly to produce for resource-poor health care systems, their therapeutic potential may never be realized. Thus, there is urgent need for development of production platforms that are cost-effective, scalable, and safe for biological therapeutics[9]. One of the most promising methods of producing proteins and other biological substances, such as antibodies and vaccines, is the use of transgenic plants that contain a gene or genes (known as the transgene) that has been artificially inserted.

Plantibodies: antibodies produced in plants

Around 1990, plants were first considered as a potential host for producing antibodies and the word "plantibody" was coined[8]. The term "plantibodies" describes the products of plants that have been genetically engineered to express antibodies and antibody fragments. With this technology, plants are being used as antibody factories (bioreactors), utilizing 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. In essence, a plantibody is an antibody produced by genetically modified plants. Antibodies, originally derived from animals, are produced in plants by transforming the latter with animal antibody genes. Although plants do not naturally make antibodies, plantibodies have been shown to function in the same way as normal antibodies[10]. This concept of using plants as heterologous expression system for recombinant antibodies (plantibodies) is now more than two decades old.

Advantages of using plants for antibody production

Plantibodies work in a similar fashion to mammalian antibodies; however, compared to conventional methods using mammalian cells, the use of plants for antibody production offers several unique advantages. Firstly, plants are widespread, abundant, and grow quickly; they usually mature after one season of growth and it is possible to bring the product to the market within a short time. Therefore, the cost of antibodies produced by plants is substantially less than that from their animal counterparts[11]. Secondly, plants are less likely to introduce adventitious human or animal pathogens compared to mammalian cells or transgenic animals, thus reducing screening costs for viruses, prions and bacterial toxins. Unlike bacterial and other prokaryotic systems, plants share a similar endomembrane system and secretory pathway with human cells[9,12]. They do not trigger immune responses which animal antibodies are prone to doing when exposed to foreign/non-self agents and they also produce a relatively high yield of antibodies in a comparatively shorter time[13]. Additionally, plants are capable of synthesizing and assembling virtually any kind of antibody molecule, ranging from the smallest antigen-binding domains and fragments to full-length and even, multimeric antibodies[14]. Plants can be engineered to produce proteins efficiently, with significantly lower manufacturing costs than mammalian cell cultures[15]. Moreover, large-scale processing infrastructure is already in place for most crops. Hence, scale-up is rapid and efficient, requiring only the cultivation of additional land[13]. Also, plants that generate large biomass like corn and tobacco can produce large amounts of genetically engineered products while proteins can be indefinitely stored on seeds with little reduction in biological activity[11].

Concerns over use of food crops for antibody production

Although plantibodies have been reported to possess many advantages, there are concerns that the purity of food crop strains could be jeopardized with fears that plants carrying antibodies could contaminate food crops or that toxin from pesticides or fertilizers could be trans-

mitted to other plants[16]. Therefore, it has been suggested in scientific literature that plants (such as tobacco or moss) that do not serve as food for people or feed for livestock, should be utilized in the production of antibodies[6]. Similarly, the concern over possible toxin transmission to food crops can be greatly reduced and almost eliminated by producing plants in contained areas such as greenhouses. In this way, plantibodies would not mix with other crop strains and the scientists could choose to forego spraying the plant hosts with pesticides. Interestingly, being contained in greenhouses would not hamper the plants' production of plantibodies as large amounts of antibodies can be extracted from relatively small plots of land. For instance, 360 million doses of plantibodies against anthrax can be produced from a single acre of tobacco[6] while 1.5 kg of antibodies is provided per acre of corn[16]. In addition, there are concerns that plants produce allergenic compounds such as protein glycans and other plant antigens[17] and those differences in codon usage between plants and prokaryotes can lead to inefficient expression of prokaryotic proteins in plants[11].

Preference of tobacco for the production of plantibodies

Several works have shown that soybeans, tobacco, potatoes, corn, alfalfa and similar crops are promising alternative for the production of recombinant therapeutic proteins[18,19,20]. Leafy crops such as tobacco and alfalfa generally have the greatest biomass yields per hectare, because they can be cropped several times a year[13]. Nonetheless, tobacco has proven to be a favorite preference as it offers numerous advantages over other plants as a host system[6,21]. Tobacco grows quickly and through numerous tests, has been shown to produce comparatively large amounts of antibodies. Additionally, tobacco is a non-food/non-feed crop, which means that if grown in a greenhouse, the use of tobacco as a host would eliminate the small chance of cross-contamination. Being a non-food/non-feed crop would also mean that the use of tobacco for antibody production would not remove any food staples from the industry or contribute in the slightest to a food shortage[10].

Methods of plantibody production

Various techniques have been developed to exploit plants as bioreactors for the production of pharmaceutical antibodies. One of the several methods for synthesizing plantibody is conventional method which uses transformation and transient expression vector to introduce new genes into a host cell. The transformant cell is then introduced into the plant embryo and propagation of the plant in the open field allows large-scale production of antibodies[10].

Plant tissue culture is the most economic and time-saving method for production of antibodies from plants. To achieve 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[20].

An experiment on tobacco plant established its breeding and sexual crossing as a method for production of plantibody. In this experiment, transformation was used to introduce kappa type of light chain into tobacco plants. The same was done with gamma heavy chains. Upon crossing one plant with kappa-chains and another plant with gamma-chains, an antibody was produced that expressed both chains[14,18].

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[22,23].

Application of plants as transgenes for biologicals

The use of transgenic plants for the expression of molecules with therapeutic, diagnostic or veterinary applications has been documented in the last decade. 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 are presently undergoing clinical trials.

i. Therapeutic applications

The first plantibody created from tobacco was called CaroRx®. It is a clinically advanced anti-*Streptococcus mutans* secretory immunoglobulin A plantibody that binds specifically to the bacterium, thus protecting humans from dental caries that the organism causes[23]. Another plantibody with human medical applications is a humanised antibody against herpes simplex virus glycoprotein B which was expressed in soybean[24].

In a study conducted by Hull et al.[25], antibodies engineered to bind to *Bacillus anthracis* were extracted from

transgenic strains of tobacco and tested in mice. The result showed that the antibodies were effective in fighting the *B. anthracis* strain and bodes well for the future if ever there is an anthrax epidemic, as there will be a cheap and effective prevention of the disease.

In a similar study, tobacco-derived plantibodies were experimentally administered in mice against the Lewis Y antigen, which is found on tumour cells in mice and in colorectal, breast, lung and ovarian cancer. The results showed that the plantibodies had a definitive positive effect on the cancer-stricken mice by preventing tumour formation in them[7]. Also, treatment or cure for rabies through plantibodies has been investigated. A plantibody-based rabies vaccine produced in tobacco was experimentally administered in hamsters to identify whether it could effectively target rabies. According to Ko et al.[26], the plantibody proved to be a safe and economically feasible alternative to the current methods of antibody production in animal systems.

Antibodies against ovarian, testicular and colon cancer as well as melanoma, B-cell lymphoma and human papillomavirus have already been expressed in transgenic tobacco[13]. These plantibodies are currently being researched and are on their way to being approved for human use. Plantibodies called DoxoRx and RhinoRx for post-cancer therapy and rhinoviruses respectively are in various stages of completion. Already, CaroRx® has been used in human trials and a tobacco plantibody against a poultry virus (Newcastle disease) has been approved by the USDA[27].

ii. Vaccination

The production of proteins in plants is a major task in producing pharmaceutical polypeptides. Potential proteins produced include cytokines, hormones, enzymes, epidermal growth factors, interferons, human protein C, and pharmaceutical food stuff which are considered for oral immunization. Transgenic plants that express antigens in their edible tissue might be used as an inexpensive oral vaccine production and delivery system. Thus, immunization might be possible through consumption of an "edible vaccine"[19], to provide active immunization. Also, plants produce different classes of proteins which are inexpensive and have increased pharmaceutical value. Due to these reasons, transgenic plants are better alternatives. Oral vaccines offer convenient immunization strategies for implementing universal vaccination programs throughout the world[28]. However, compared to vaccines, plantibodies have one major demerit - the introduced antibodies are flushed through a person's system relatively quickly, in a matter of hours or days, before the host's immune system has adapted to producing antibodies. Furthermore, vaccines elicit antibody production so that one or a few doses can protect the individual for year(s). By contrast, if a plantibody is being used to prevent a disease, the patient would need to take doses indefinitely. Other disadvantages of adoption of antibody expression in plants include gene silencing in some instances, different patterns of glycosylation, insufficient expression in some plants, allergies or allergic reactions to plant glycoproteins and other plant antigens[29].

iii. Immunomodulation

Immunomodulation is a molecular technique that allows the interference with cellular metabolism or pathogen infectivity by the ectopic expression of genes encoding antibodies or antibody fragments[30]. Applications relying on modulating antigen levels in vivo are dependent on expression and accumulation of antibodies in specific subcellular compartments and 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 [30,31]. Additionally, agro-infiltration of tobacco has been used to produce a diabody against carcinoembryonic antigen[32]. Other than applications in human healthcare, plantibodies may also prove useful as feed additives or for phytoremediation[19].

iv. Contemporary application: treatment of Ebola patients

The production of anti-Ebola virus antibodies has recently been explored in plants. Chen et al.[33] used a high-yielding geminivirus-based expression system in the tobacco plant, *Nicotiana benthamiana*, for the production of a mAb (6D8) that protected animals from Ebola virus infection. Using similar technology and *N. benthamiana*, Bhoo et al.[34] produced an Ebola immune complex (EIC) by fusing Ebola envelope glycoprotein GP1 to the C-terminus of the heavy chain (HC) of humanized 6D8 mAb that binds specifically to a linear epitope on GP1. Geminivirus vector-mediated co-expression of the GP1-HC fusion and the 6D8 light chain in *N. benthamiana* leaves produced assembled immunoglobulin, which was purified by protein G affinity chromatography. The resultant recombinant antibody bound the complement factor C1q, indicating immune complex formation. Thereafter,

subcutaneous immunization of mice with purified EIC elicited high level production of anti-Ebola virus antibodies. This was the first published account of an Ebola virus candidate vaccine to be produced in plants.

The ongoing outbreak of Ebola virus disease in West Africa has provided a unique opportunity for the use of plantibodies in solving global human health challenges as two American medical aid workers who contracted the disease in Liberia were successfully treated with an experimental drug produced in the tobacco plant. The drug, called ZMapp, contains a cocktail of three humanized anti-Ebola virus mAbs and was developed by Mapp Biopharmaceutical Incorporated, San Diego[35]. However, although ZMapp holds great promise for the future, a major factor that contributed to its limited use in the ongoing West African Ebola outbreak is that producing the huge quantity of anti-Ebola virus antibody cocktail needed on a global scale would be challenging as, in its current form, ZMapp therapy requires the delivery of multiple doses of highly pure antibody directly into the bloodstream. Consequently, it would be difficult to meet the antibody demand for widespread use in Ebola virus-stricken African countries. Moreover, the drug was originally intended for expression at levels sufficient for animal trial use[36]. In addition, ZMapp is yet to receive approval by the US Food and Drug Administration who would have to certify, for instance, that the plant extraction process has not led to contamination of the resulting drug[37].

Conclusion

Transgenic plants have been shown to be the most productive and economical system for making antibodies for human use as they play a key role in providing therapeutics and edible vaccines, which are cheap and easy to administer. This is true considering that not only tobacco, but also many other common plants such as corn, moss and soybeans have become hosts for antibodies and have the capacity to cure, treat or lessen the detrimental effects of multiple diseases. The low-cost, high-scalability, and safety characteristics of a plant-based production system offer an attractive alternative for both commercial pharmaceutical production and for manufacturing products for the developing world[9]. Furthermore, adoption of plants as bioreactors on a larger scale would reduce the cost of antibody therapy and increase the number of patients with access to these treatments[11]. In light of their numerous advantages, it seems likely that plantibodies are the potential panacea for human and animal health challenges in the foreseeable future. As their use in solving human health problems seem to be increasing, we advocate that their application should also be exploited in the field of veterinary medicine. Lastly, this important biotechnological breakthrough should be embraced in Africa where there is great diversity of crops and plants that can be readily explored by the pharmaceutical industry for therapeutic, immunoprophylactic, improved livestock productivity and other purposes.

Conflict of interest

The authors declare that no potential conflict of interest exists in preparing this manuscript.

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