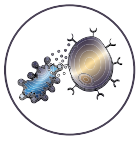
Marconi and Alvarez, J Immunol Tech Infect Dis 2014, 3:2
http://dx.doi.org/10.4172/2329-9541.1000125



Journal of Immunological Techniques in Infectious Diseases

Review Article

A SCITECHNOL JOURNAL

State of the Art on Plant-Made Single-Domain Antibodies

Patricia Marconi[1] and Maria A Alvarez[1]*

Abstract

In addition to conventional antibodies (with heavy and light chains), camelids also produce functional antibodies devoid of light chains (HCAbs) without the first constant domain (CH1). Their variable domains (VHH) have binding properties, high stability and solubility, and are considered the smallest available intact antigen–binding fragment derived from a functional immunoglobulin. For their practical utilities VHHs have been expressed in different platforms. This review aims to provide an update in the field of plant-made VHHs, their applications and limitations, and a discussion about the challenges for the near future in this field.

Keywords

VHHs; Single-domain antibodies; Camelid antibodies; Plant-made proteins

Introduction

Antibodies are one of the most powerful tools in therapy and diagnostics and also one of the fastest growing classes of therapeutic molecules [1]. Monoclonal antibodies, which are used to treat a wide range of diseases, were made possible by the discovery of how to generate a continuous culture of hybridoma cells secreting antibodies of predefined specificity [2]. As recombinant antibodies are molecules with a remarkable demand by the medical industry, a broad range of biological systems is being used for their production, such as bacteria, transgenic animals, transgenic plants, tissue cultures, etc. The choice of the right antibody format depends on their biotechnological or therapeutic potential use [3–6].

Recently, a group of small antibodies are concentrating the interest of the pharmaceutical industry for their particular properties, the heavy-chain antibodies (HCAbs) of Camelidae (camels, dromedaries, guanacoes, alpacas, vicugnas, and llamas) [7]. In addition to conventional antibodies (with heavy and light chains), camelids also contain antibodies with solely the heavy chain of an ordinary immunoglobulin without the first constant domain (CH1) and with aminoacids substitutions involving conserved hydrophobic residues [8,9] (Figure 1). The variable domain (VHH) of those HCAbs has binding properties and is considered as the smallest available intact antigen–binding fragment (approximately 15 kDa) derived from a functional immunoglobulin [10,11].

The Variable Domain of the Heavy-Chain Antibodies of Camelidae

The VHHs, also called nanobodies™ (Ablynx) or domain antibodies (dAbs), can be cloned from the blood lymphocytes of an immunized dromedary and could also be produced as recombinant proteins in bacteria, yeast and plants. They appear to be quite soluble, without the tendency to aggregate, with an affinity in the same range as that of scFv fragments, and are resistant to the action of proteases. Besides, the VHHs could be directed toward epitopes that are nonimmunogenic for conventional antibodies, such as the active site of enzymes, resulting in potent enzyme inhibitors with the ability of recognizing or having access to structures that are not recognized or are inaccessible to conventional antibodies. Also, inducing multimerization with genetically encoded linkers or carriers could increase their potency. The unique biochemical and biophysical properties previously described make VHHs attractive to be produced as recombinant molecules with numerous applications [12,13].

A broad amount of information is available regarding the production of VHHs in different platforms [13,14]. The aim of this paper is to summarize the most recent articles about plant-made VHHs, and their applications and limitations.

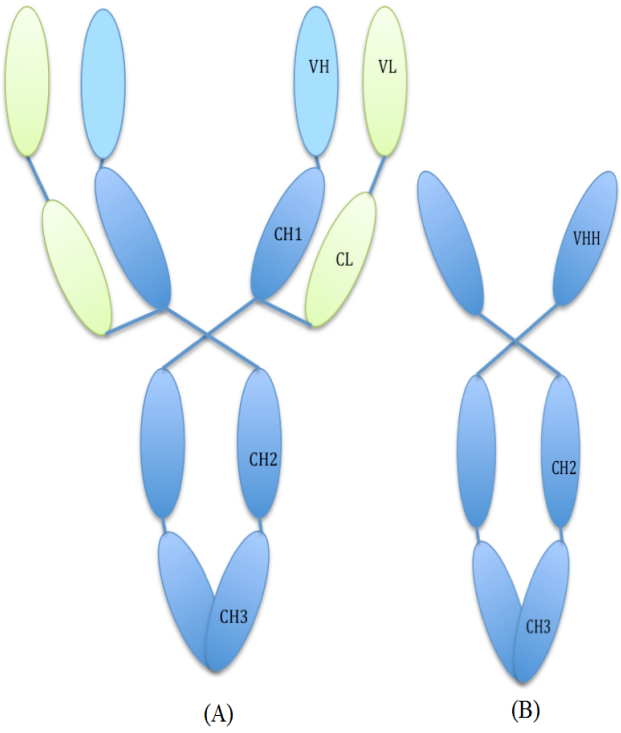


Figure 1: Structure of convencional (A) and camelid derived heavy chain antibody (B). H: heavy chain, L: Light chain, C: constant domain, V: variable domain, VHH: variable domain of heavy chain of heavy-chain antibody.

***Corresponding author:** Maria A Alvarez, CONICET/ School of Pharmacy and Biochemistry, Universidad Maimónides, Hidalgo 775 (1404), 6th floor, Ciudad de Buenos Aires, Argentina, E-mail: alvarez.mariaalejandra@maimonides.edu

Received: February 26, 2014 **Accepted:** March 31, 2014 **Published:** April 04, 2014

Plants as a Productive Platform of Recombinant Antibodies

Recombinant proteins are produced in traditional platforms as mammalian and microorganism cultures. As the requirement of recombinant proteins is continuously growing, there is a shortage of sources that is intended to be covered by insect cell cultures, and transgenic plants and animals. All platforms present some favourable and unfavourable aspects. Particularly, in the case of plant-based systems, which are the topic of this review, two remarkable advantages over the alternative production platforms are their ability to carry out the post-translational modifications that are not available in bacterial systems, as well as their higher safety when compared to animal-based systems [15]. The first whole plant-made antibody reported was the catalytic IgG1-antibody type 6D4, which was expressed in *Nicotiana tabacum* [16]. Since then, many antibodies and their fragments have been produced in plants with multiple applications such as animal and human therapeutics, diagnostic, purification, etc. [14,17]. Also, several approaches have been made regarding plant-expression systems (whole plant, *in vitro* cultures), transformation methods (viral vectors, *Agrobacterium*, magnification), and subcellular targeting (endoplasmic reticulum, plastids). If the antibody has to be used for therapy, diagnosis or prevention it must be properly folded and assembled which implies a correct formation of disulphide bonds and glycosylation [18]. Glycosylation influences vital biological characteristics such as stability, immunogenicity/allogenicity, and ligand-receptor protein interactions. Usually for proper folding and glycosylation the antibody synthesis is targeted to the secretory path. Also, several glyco-engineering strategies have emerged for the tailor making of N-glycosylation in plants, including glycoprotein subcellular targeting, the inhibition of plant specific glycosyltranferases, or the addition of human specific glycosyltransferases [3]. When glycosylation is not needed, transgene integration is targeted to plastids, e.g.: chloroplasts, for avoiding gene silencing, increasing yields and avoiding environmental concerns [19,20]. A brief list of some of the antibodies and antibody fragments expressed plant systems is shown in Table 1.

Table 1: Some antibodies and antibody fragments expressed in plant systems

Antibody	Plant species	Antibody-type	Reference
6D4	*N. tabacum*	IgG_1-like	[44]
MAK33	*N. tabacum*	IgG	[45]
MAK33	*A. thaliana*	IgG, Fab	[46,47]
Anti-atrazine Anti-paraquat	*N. tabacum*	scFv	[48]
Anti-corn stutn spiroplasma	*Maize*	scFv	[49]
Bisc Fv2429	*N. tabacum (plant and suspended cells)*	biscFv	[50]
Anti-ErbB-2	*N. tabacum*	scFv	[51]
Anti-TMV	*N. tabacum*	IgG	[52]
rAb29 scFv29	*N. tabacum*	IgG scFv	[53]
Anti-HBsAg	*N. tabacum*	scFv	[54]
14D9	*N. tabacum*	IgG	[55,56]
Anti CDC2a	*A. thaliana*	scFv	[57]
Guy's 13	*N. tabacum*	IgG	[58]
Anti PVX	*N. benthamiana, Solanum esculentum, Datura stramonium, Chenopodium amaranticolor, C. quinoa, N. clevelandii*	scFv	[59]
CaroRx	*N. tabacum*	SIgA	[60]
Anti-Rhesus D	*A. thaliana*	IgG1	[61]
Anti human spleen ferritin	*N. tabacum*	scFv	[62]
Anti-HCG	*N. tabacum*	scFv	[63]
C5-1	*Alfalfa*	IgG	[64]
Anti-solasodine	*Solanum khasianum*	scFv	[65]
TheraCIM(anti-EGF receptor)	*N. tabacum*	IgG	[66]
hOAT	*Lactuca sativa L.*	IgG1	[67]
Anti-rabies virus	*N. tabacum cv. Xanthi*	IgG	[68]
Anti-CD4 and anti-CD28	*Wheat var. Westonia*	scFv	[69]
Anti- MUC1	*N. tabacum cv Xanthi*	scFv	[24]
Anti-K99	*Oryza sativa*	scFv	[70]
Hu-E16	*N. benthamiana*	IgG	[71]
mAb H10	*N. benthamiana*	IgG	[72]

Recombinant VHHs in Plants

There is a great amount of information about the production of VHHs in several platforms [10] but there are not as many reports related to plants. Up to date, VHHs were expressed for displaying its activity *in planta*, as immunomodulators of enzyme activity, and *ex planta*, for molecular farming [21,22]. A brief list of plant-made VHHs is shown in Table 2. We will focus our review mainly in VHHs expressed for *ex planta* purpose.

VHH expression for *in planta* activity

The expression of a VHH that inhibits the potato starch branching enzyme A (SBE A) function in *Solanum tuberosum* was reported by Jobling et al. in 2003 [22]. The VHH fragments with strong binding to SBE A were selected from a single-domain llama antibody fragment library using phage display technology. Those fragments, fused to the targeting peptide of the granule-bound starch synthase (GBSS), were targeted to *S. tuberosum* plastids (chloroplasts in leaf and amyloplasts in tubers). A strong correlation between high amylose content of the starch and the reduction in SBE activity was demonstrated in the immunomodulated lines. Besides, they have found that the VHH antibody fragments expressed in other cellular compartments also inhibit enzyme activity for not depending on interchain interactions for stability. Moreover, the antibody-mediated inhibition of enzyme activity obtained was more efficient than when they had tested antisense technology [23]. Remarkably, chloroplasts in the transformed plants showed a normal morphology. They have concluded that camelid single-domain antibodies are particularly useful when a neutralizing antibody is required.

Expression for *ex planta* purpose

Stable expression: Stable expression involves the introduction of the foreign gene/s to the plant nuclear or plastid genome.

Nuclear transformation: Ismaili et al. [24] reported the expression of a recombinant VHH antibody fragments against MUC1 tobacco plants. MUC1 is a transmembrane MUC1 molecule that is expressed at the luminal surface of most simple epithelial cells in normal

Table 2: Some VHHs expressed in plant systems.

VHH	Plant species	Activity	Reference
MUC1-VHH	*N. tabacum*	Anti-MUC1n	[15]
MUC1-VHH	*N. tabacum*	Anti-MUC1n	[24]
MUC1-VHH	*N. tabacum*	Anti-MUC1n	[28]
Anti-HEWL-VHH	*N. benthamiana*	Anti-hen egg white lysozyme	[37]
Anti-TNFα VHH	*A. thaliana*	Anti-tumour necrosis factor	[73]
Anti-SBE A VHH	*S. tuberosum*	Inhibition of SBE A enzyme	[22]
Anti-tetanus toxin VHH	*N. tabacum*	Inhibitory effect	[33]
VHH7-Fc	*A. thaliana*	Anti- prostate specific antigen	[32]
3B2-VHH	*N. tabacum*	Anti-rotavirus VP6 protein	[36]
anti- TNFh-VHH	*N. tabacum*	Blocking effect of the Tumour Necrosis Factor (NFh)	[29]
VHH-IgG and VHH-IgA anti-ETEC	*A. thaliana*	Protection against against the piglet postweaning diarrhea	[30]
VHH anti-rotavirus (MucoRice)	*Oryza sativum*	Protection against rotavirus in mice	[31]
VHH	*N. benthamiana*	anti-human TNF-alpha	[74]

cases. Usually, its expression increased during lactation and in most breast carcinoma. In breast cancer the profile of glycotransferases involved in the O-glycosylation of MUC1 changed which makes MUC1 potentially useful as tumour-associated antigen and as target antigen for immunotherapy [25]. Although a VHH anti-MUC1 was already expressed in *E. coli* and *P. pastoris* the limitations of microbial expression systems moved the authors to the plant platform. They constructed the pBI-VHH plant binary vector containing the CaMV35S promoter, and the NOS terminator and performed *Agrobacterium*-mediated transformation of tobacco plants. The yield of purified MUC1-VHH was 28-136 µg/g of fresh leaf weight, whereas in bacteria and yeast was 5 and 10 mg l^{-1} respectively [26,27].

The immunological activity of the plant-made MUC1-VHH, determined by an ELISA, assay was higher than that of VHH from bacteria and similar to that of yeasts, whereas when the reactivity was measured by immunocytochemistry it was higher in plants than in the two microbial systems. Those studies have confirmed that the plant-made MUC1-VHH can be used as a targeting reagent for recognition of the MUC1 tandem region on the surface of cancer cells. A previous report related to the expression of a VHH against MUC1 MUC1n in tobacco was published in 2006 [28]. The VHH gene was introduced into tobacco by *Agrobacterium*-mediated transformation with expression levels varying from 1.12 to 1.63 % of the total soluble protein content.

Korouzhdehy et al. [15] have also described the cloning and expression of a MUC1 VHH single domain antibody in other variety of tobacco plants (*N. tabacum NC25*). They have used the pBI-VHH plasmid with the CaMV 35S promoter, and the T/A cloning vector (PTZ57R) to take advantage of multiple cloning sites. Transformation was made using *A. tumefaciens* (strains LBA4404 and C58GV3101). The regenerated plants expressed the MUC1-VHH without any effect on plant growth. The VHH accumulated in the cytosol of plant cells maintaining its functionality. Furthermore, they have validated an ELISA that is not affected by impurities of the sample for the quantification of anti-MUC1-VHH. Tobacco plants were also the production platform used for a VHH anti-human tumour necrosis factor (TNF, a major pro-inflammatory cytokine) with the aim of evaluating its potential as a TNF antagonist [29]. The fusion protein comprised the corresponding VHH linked to an elastin-like polypeptide (ELP) resulting an heterologous protein with biological activity *in vivo* which was effective in preventing death caused by septic shock in mice. An interesting work was performed comparing the accumulation of two camelid recombinant VHH antibodies in a variety of plant cell compartments [6]. The anti-TNFα VHH coding sequences, the fused tandem heterodimers, and a tandem fusion of two VHH (G7 and F4) were placed in cassettes designed to target the protein to the cytoplasm, endoplasmic reticulum, protein storage vacuole or chloroplast. Although expression was achieved in all cases the combination of ER targeting with the G7:F4 fusion let to the highest accumulation of the anti-TNFα VHH in the leaf. When the *in vitro* antigen binding capacity and functional stability in rumen fluid between VHHs expressed in plants and in prokaryotic cells was compared, differences in stability were observed. However, plant-made VHHs were able to bind equally well to the antigen in the presence or absence of rumen fluid [6].

Recently, in order to analyze the possibility of achieving an oral passive immunization against the piglet postweaning diarrhea (PWD) caused by enterotoxigenic *Escherichia coli* (ETEC), several anti-ETEC antibodies were designed by fusing the VHHs against ETEC to the Fc part of a porcine immunoglobuline (IgG and IgA). The VHH brings its resistance to extreme conditions (pH, temperature) and its ability to interact with deep antigenic clefts by means of a loop in the C3 domain, and the fusion with Fc ensure multiple valences to the chimeric divalent antibody. Four VHH-FcIgG and four VHH-FcIgA were obtained, which were expressed in *Arabidopsis thaliana* seeds with expression levels of 3 % for VHH-FcIgG and 0.2 % for VHH-FcIgA. Furthermore, cotransformation of VHH-IgA with the J chain- and SC-produced multivalent dVHH-IgAs and sVHH-IgAs. Seeds expressing all the chimeric antibodies were used to elaborate feed formulations for piglets with different antibody concentration. Interestingly, only piglets receiving the VHH-FcIgA (20 mg/d per pig) in the feed were protected. Among other hypothesis, the authors refer that the stability of antibody constructs in gut might have favoured efficacy of VHH-IgA-20 feed over VHH-IgG-80 feed. Future experiments expressing the chimeric antibodies in feed crops (soybean or pea) are planned to estimate the final cost of the product and to define if it would be economically attractive to implement this oral passive immunization [30].

On the other hand, the variable domain of a rotavirus-specific llama-heavy chain antibody against rotavirus was expressed in rice seeds using the MucoRice technology [31]. The internal storage protein production was suppressed by introducing RNAi to the anti rotavirus-VHH (ARP1) production system and by co-introducing antisense genes specific for the prolamin and glutelin rice storage proteins. The expression levels attained were high (11.9% of total protein) with the advantages that, as it is expressed in seeds, there are no need for purification, nor cold-chain transport and storage. The oral administration of MucoRice-ARP1 afforded protection against rotavirus in mice. Recently, VHH-Fc fusions were expressed in *A. thaliana* seeds in order to analyze the effect on normal cell homeostasis of protein production in the endoplasmic reticulum. They theorized that the accumulation in the ER lead to an ER stress response named UPR which eventually could conduct to a plant apoptotic programme cell death [32]. Also they suggest a correlation between the properties of the expressed proteins and the ER induced stress.

Plastid transformation: A pioneer work was carried out by Vü

[33] that reported the expression of a camel VHH anti-tetanus toxoid gene in N. tabacum targeted to the chloroplasts by a fusion with an N-terminal chloroplast-targeting peptide. Total leaf extracts rendered VHH levels up to 0.1 % of the total soluble protein (TSP) content, but they were not detected in chloroplasts extracts. Also, a VHH (AbL) that binds to an antigen-chicken egg-white lysozyme was expressed in *N. tabacum* plastid genome. The expression cassette also bears the T7 promoter and the T7RNAP system to activate the AbL gene expression in the transplastomic line [34]. VHH expression resulted semi-lethal to the progeny although further research has associated those results to the use of the T7RNAP strategy. Apparently the T7RNAP-mediated transcription of plastid transgenes can disrupt both the transcription of plastid transgenes and the expression of nuclear genes encoding plastid proteins [35]. Another example is the expression of the 3B2 VHH antibody directed against the rotavirus VP6 protein in *A. thaliana*. The VHH expression was directed to the chloroplast stroma, using the construct pBSW-utr/VHH, or into the thylakoid lumen replacing the signal peptide of the former construction by one from the N-terminal sequence of the pectate lyase B of *Erwinia carotovora* (pep-VHH). A third construction tested resulted from the 3B2 VHH fusion to the complete sequence of the β–glucuronidase enzyme gene (GUS-E-VHH). No success was attained in the attempt to express VHH 3B2 in the chloroplast stroma. On the other side, higher levels were detected in the case of pep-VHH (2-3 % of the total soluble proteins) and GUS-E-VHH (3% of the total soluble proteins). The activity of pep-VHH and GUS-E-VHH was demonstrated by ELISA with human rotavirus Wa-captured plates and by a virus neutralization assay. The sensitivity of the ELISA test was up to 15.64 ng of recombinant protein per well and the purified protein obtained from pep-VHH was able to neutralize the bovine rotavirus as strongly as the VHH produced in *E. coli*. GUS-E-VHH did not show neutralizing activity at the concentrations tested. However, the expression of those VHHs in transplastomic tobacco plants resulted in the production of degradation products or a chlorotic phenotype [36].

Transient expression: In this case the foreign gene expression does not depend on chromosomal integration, it is delivered into the nucleus and remains transcriptionally competent for several days. As other recombinant proteins, camelid nanobodies were also expressed transiently in plants. For example, the three versions of an anti-hen egg white lysozyme (HEWL) VHH in *N. benthamiana* using a 'deconstructed' viral expression system [37]. His6- and StrepII-tagged derivatives of each nanobody were targeted to the cytoplasm, chloroplast and apoplast [38-41]. The fully functional nanobodies™ accumulated at different levels according to their place of accumulation (e.g.: up to 30% total leaf protein in the apoplast of agroinfiltrated leaves) and the framework upon each version is based.

Conclusion

VHHs, which are the smallest fully active antibody fragment have unique biophysical and pharmacological properties that make them particularly promising for medicine (research, diagnostics and therapy) and biotechnology. Plants have already demonstrated to be an efficient protein production platform, and several strategies have been used to improve the performance of stable expressed VHHs (e.g.: transient transformation, localization of the recombinant VHH in subcellular compartments). Those strategies are time-consuming but it can be compensated by the lack of animal or human pathogen contaminating the product, or by the simplicity of the downstream processing that considerably diminishes costs. It was reported that recombinant proteins could be produced in plants at 2 to 10 % of the cost of microbial fermentation systems and at 0.1 % of mammalian cell cultures depending on product yield [42,43]. A drawback to using plant-made proteins to human health is the different glycosilation patterns of mammal and plant proteins. That problem can be overcome by "glycosilation engineering" that has resulted successful in adapting plant-made antibodies. A milestone in plant molecular farming is the FDA approval of taliglucerase alfa, a plant-derived glucocerebrosidase, that was produced in carrot root cells by Protalix Biotherapeutics and distributed by Pfizer, Inc. under the trade name ELELYSO™ (UPLYSO™) for the treatment of Gaucher's disease [75]. It is expected that more developments will be communicated in the short time promoting the application of plant-made proteins, and particularly VHHs considering their multiple potential applications.

Acknowledgements

María Alejandra Alvarez and Patricia Laura Marconi belong to the National Council of Research from Argentina (CONICET). This paper was supported by the National Agency of Science, Technology and Productive Innovation from Argentina (ANPCyT, PICT2007, N°00552).

References

1. Bernal AJ, Willats WG (2004) Plant science in the age of phage. Trends Plant Sci 9: 465-468.
2. Elvin JG, Couston RG, van der Walle CF (2013) Therapeutic antibodies: market considerations, disease targets and bioprocessing. Int J Pharm 440: 83-98.
3. De Muynck B, Navarre C, Boutry M (2010) Production of antibodies in plants: status after twenty years. Plant Biotechnol J 8: 529-563.
4. Galeffi P, Lombardi A, Pietraforte I, Novelli F, Di Donato M, et al. (2006) Functional expression of a single-chain antibody to ErbB-2 in plants and cell-free systems. J Transl Med 4: 39.
5. Galeffi P, Lombardi A, Donato MD, Latini A, Sperandei M, et al. (2005) Expression of single-chain antibodies in transgenic plants. Vaccine 23: 1823-1827.
6. Winichayakul S, Pernthaner A, Livingston S, Cookson R, Scott R, et al. (2012) Production of active single-chain antibodies in seeds using trimeric polyoleosin fusion. J Biotechnol 161: 407-413.
7. Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, et al. (1993) Naturally occurring antibodies devoid of light chains. Nature 363: 446-448.
8. De Simone E, Saccodossi N, Ferrari A, Leoni L, Leoni J (2006) Immunochemical analysis of IgG subclasses and IgM in South American camelids. Small Rumin Res 64: 2-9.
9. Conrath KE, Wernery U, Muyldermans S, Nguyen VK (2003) Emergence and evolution of functional heavy-chain antibodies in Camelidae. Dev Comp Immunol 27: 87-103.
10. Muyldermans S, Cambillau C, Wyns L (2001) Recognition of antigens by single-domain antibody fragments: the superfluous luxury of paired domains. Trends Biochem Sci 26: 230-235.
11. Thomassen YE, Verkleij AJ, Boonstra J, Verrips CT (2005) Specific production rate of VHH antibody fragments by Saccharomyces cerevisiae is correlated with growth rate, independent of nutrient limitation. J Biotechnol 118: 270-277.
12. de Marco A (2011) Biotechnological applications of recombinant single-domain antibody fragments. Microb Cell Fact 10: 44.
13. Vanlandschoot P, Stortelers C, Beirnaert E, Ibañez LI, Schepens B, et al. (2011) Nanobodies®: new ammunition to battle viruses. Antiviral Res 92: 389-407.
14. Tillib SV (2011) Camel nanoantibody" is an efficient tool for research, diagnostics and therapy. Mol Biol (Mosk) 45: 77-85.
15. Korouzhdehy B, Dadmehr M, Piri I, Rahbarizadeh F, Solouki M (2011) Expression of biological active VHH camelid single domain antibody in transgenic tobacco. Afr J Biotechnol 10: 4234-4241.

16. De Muynck B, Navarre C, Boutry M (2010) Production of antibodies in plants: status after twenty years. Plant Biotechnol J 8: 529-563.

17. Thomas DR, Penney CA, Majumder A, Walmsley AM (2011) Evolution of plant-made pharmaceuticals. Int J Mol Sci 12: 3220-3236.

18. Ko K, Koprowski H (2005) Plant biopharming of monoclonal antibodies. Virus Res 111: 93-100.

19. Daniell H, Chebolu S, Kumar S, Singleton M, Falconer R (2005) Chloroplast-derived vaccine antigens and other therapeutic proteins. Vaccine 23: 1779-1783.

20. Meyers B, Zaltsman A, Lacroix B, Kozlovsky SV, Krichevsky A (2010) Nuclear and plastid genetic engineering of plants: comparison of opportunities and challenges. Biotechnol Adv 28: 747-756.

21. De Jaeger G, De Wilde C, Eeckhout D, Fiers E, Depicker A (2000) The plantibody approach: expression of antibody genes in plants to modulate plant metabolism or to obtain pathogen resistance. Plant Mol Biol 43: 419-428.

22. Jobling SA, Jarman C, Teh MM, Holmberg N, Blake C, et al. (2003) Immunomodulation of enzyme function in plants by single-domain antibody fragments. Nat Biotechnol 21: 77-80.

23. Schwall GP, Safford R, Westcott RJ, Jeffcoat R, Tayal A, et al. (2000) Production of very-high-amylose potato starch by inhibition of SBE A and B. Nat Biotechnol 18: 551-554.

24. Ismaili A, Jalali-Javaran M, Rasaee MJ, Rahbarizadeh F, Forouzandeh-Moghadam M, et al. (2007) Production and characterization of anti-(MUC1n MUC1) single-domain antibody in tobacco (Nicotiana tabacum cultivar Xanthi). Biotechnol Appl Biochem 47: 11-19.

25. Taylor-Papadimitriou J, Burchell J, Miles DW, Dalziel M (1999) MUC1 and cancer. Biochim Biophys Acta 1455: 301-313.

26. Rahbarizadeh F, Rasaee MJ, Forouzandeh M, Allameh AA (2006) Over expression of anti-MUC1 single-domain antibody fragments in the yeast Pichia pastoris. Mol Immunol 43: 426-435.

27. Rahbarizadeh F, Rasaee MJ, Forouzandeh-Moghadam M, Allameh AA (2005) High expression and purification of the recombinant camelid anti-MUC1 single domain antibodies in Escherichia coli. Protein Expr Purif 44: 32-38.

28. Rajabi-Memari H, Jalali-Javaran M, Rasaee MJ, Rahbarizadeh F, Forouzandeh-Moghadam M, et al. (2006) Expression and characterization of a recombinant single-domain monoclonal antibody against MUC1 MUC1n in tobacco plants. Hybridoma (Larchmt) 25: 209-215.

29. Conrad U, Plagmann I, Malchow S, Sack M, Floss DM, et al. (2011) ELPylated anti-human TNF therapeutic single-domain antibodies for prevention of lethal septic shock. Plant Biotechnol J 9: 22-31.

30. Virdi V, Coddens A, De Buck S, Millet S, Goddeeris BM, et al. (2013) Orally fed seeds producing designer IgAs protect weaned piglets against enterotoxigenic Escherichia coli infection. Proc Natl Acad Sci U S A 110: 11809-11814.

31. Tokuhara D, Álvarez B, Mejima M, Hiroiwa T, Takahashi Y, et al. (2013) Rice-based oral antibody fragment prophylaxis and therapy against rotavirus infection. J Clin Invest 123: 3829-3838.

32. De Wilde K, De Buck S, Vanneste K, Depicker A (2013) Recombinant antibody production in Arabidopsis seeds triggers an unfolded protein response. Plant Physiol 161: 1021-1033.

33. Vü KB (1999) Llama antibodies and engineering of single-domain antibody fragments. Vrije Universiteit Brussel, Brussels, Belgium.

34. Magee AM, Coyne S, Murphy D, Horvath EM, Medgyesy P, et al. (2004) T7 RNA polymerase-directed expression of an antibody fragment transgene in plastids causes a semi-lethal pale-green seedling phenotype. Transgenic Res 13: 325-337.

35. Magee AM, MacLean D, Gray JC, Kavanagh TA (2007) Disruption of Essentials plastid gene expresión caused by T7 RNA polymerase-mediated transcription of plastid transgenes during early seedling development. Transgenic Res 16: 415-428.

36. Lentz EM, Garaicoechea L, Alfano EF, Parreño V, Wigdorovitz A, et al. (2012) Translational fusion and redirection to thylakoid lumen as strategies to improve the accumulation of a camelid antibody fragment in transplastomic tobacco. Planta 236: 703-714.

37. Teh YH, Kavanagh TA (2010) High-level expression of Camelid nanobodies in Nicotiana benthamiana. Transgenic Res 19: 575-586.

38. Arbabi Ghahroudi M, Desmyter A, Wyns L, Hamers R, Muyldermans S (1997) Selection and identification of single domain antibody fragments from camel heavy-chain antibodies. FEBS Lett 414: 521-526.

39. Marillonet S, Giritch A, Gils M, Kandzia R, Klimyuk V, et al. (2004) In planta engineering of viral RNA replicons: efficient assembly by recombination of DNA modules delivered by Agrobacterium. Proc Natl Acad Sci USA 101: 6852-6857.

40. Saerens D, Pellis M, Loris R, Pardon E, Dumoulin M, et al. (2005) Identification of a universal VHH framework to graft non-canonical antigen-binding loops of camel single-domain antibodies. J Mol Biol 352: 597-607.

41. Giritch A, Marillonnet S, Engler C, van Eldik G, Botterman J, et al. (2006) Rapid high-yield expression of full-size IgG antibodies in plants coinfected with noncompeting viral vectors. Proc Natl Acad Sci U S A 103: 14701-14706.

42. Sala F, Manuela Rigano M, Barbante A, Basso B, Walmsley AM, et al. (2003) Vaccine antigen production in transgenic plants: strategies, gene constructs and perspectives. Vaccine 21: 803-808.

43. Rybicki EP (2009) Plant-produced vaccines: promise and reality. Drug Discov Today 14: 16-24.

44. De Muynck B, Navarre C, Boutry M (2010) Production of antibodies in plants: status after twenty years. Plant Biotechnol J 8: 529-563.

45. Bruyns AM, De Jaeger G, De Neve M, De Wilde C, Van Montagu M, et al. (1996) Bacterial and plant-produced scFv proteins have similar antigen-binding properties. FEBS Lett 386: 5-10.

46. De Wilde C, De Neve M, De Rycke R, Bruyns A, De Jaegger G, et al. (1996) Intact antigen-binding MAK33 antibody and Fab fragment accumulate in intercellular spaces of Arabidopsis thaliana. Plant Science 114: 233-241.

47. Peeters K, De Wilde C, Depicker A (2001) Highly efficient targeting and accumulation of a F(ab) fragment within the secretory pathway and apoplast of Arabidopsis thaliana. Eur J Biochem 268: 4251-4260.

48. Longstaff M, Newell CA, Boonstra B, Strachan G, Learmonth D, et al. (1998) Expression and characterisation of single-chain antibody fragments produced in transgenic plants against the organic herbicides atrazine and paraquat. Biochim Biophys Acta 1381: 147-160.

49. Chen YD, Chen TA (1998) Expression of engineered antibodies in plants: a possible tool for spiroplasma and phytoplasma disease control. Phytopathology 88: 1367-1371.

50. Fischer R, Liao YC, Drossard J (1999) Affinity-purification of a TMV-specific recombinant full-size antibody from a transgenic tobacco suspension culture. J Immunol Methods 226: 1-10.

51. Galeffi P, Lombardi A, Donato MD, Latini A, Sperandei M, et al. (2005) Expression of single-chain antibodies in transgenic plants. Vaccine 23: 1823-1827.

52. Fischer R, Schumann D, Zimmermann S, Drossard J, Sack M, et al. (1999) Expression and characterization of bispecific single-chain Fv fragments produced in transgenic plants. Eur J Biochem 262: 810-816.

53. Schillberg S, Zimmermann S, Voss A, Fischer R (1999) Apoplastic and cytosolic expression of full-size antibodies and antibody fragments in Nicotiana tabacum. Transgenic Res 8: 255-263.

54. Ramírez N, Lorenzo D, Palenzuela D, Herrera L, Ayala M, et al. (2000) Single-chain antibody fragments specific to the hepatitis B surface antigen, produced in recombinant tobacco cell cultures. Biotechnology Letters. 22: 1233-1236.

55. Martínez C, Petruccelli S, Giulietti AM, Alvarez MA (2005) Expression of the antibody 14D9 in Nicotiana tabacum hairy roots. Electron J Biotechnol 8: 170-176.

56. Petruccelli S, Otegui MS, Lareu F, Dinh T, Fitchette AC, et al. (2006) A KDEL-tagged monoclonal antibody is efficiently retained in the endoplasmic reticulum in leaves, but is both partially secreted and sorted to protein storage vacuoles in seeds. Plant Biotechnol J 4: 511-527.

57. Eeckhout D, Fiers E, Sienaert R, Snoeck V, Depicker A, et al. (2000) Isolation and characterization of recombinant antibody fragments against CDC2a from Arabidopsis thaliana. Eur J Biochem 267: 6775-6783.

58. Sharp JM, Doran PM (2001) Strategies for enhancing monoclonal antibody accumulation in plant cell and organ cultures. Biotechnol Prog 17: 979-992.

59. Roggero P, Ciuffo M, Benvenuto E, Franconi R (2001) The expression of a single-chain Fv antibody fragment in different plant hosts and tissues by using Potato virus X as a vector. Protein Expr Purif 22: 70-74.

60. Ma JK, Hikmat BY, Wycoff K, Vine ND, Chargelegue D, et al. (1998) Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. Nat Med 4: 601-606.

61. Bouquin T, Thomsen M, Nielsen LK, Green TH, Mundy J, et al. (2002) Human anti-rhesus D IgG1 antibody produced in transgenic plants. Transgenic Res 11: 115-122.

62. Semenyuk EG, Orlova IV, Stremovskii OA, Balandin TG, Nosov AM, et al. (2002) Transgenic tobacco plants produce miniantibodies against human ferritin. Dokl Biochem Biophys 384: 176-178.

63. Kathuria S, Sriraman R, Nath R, Sack M, Pal R, et al. (2002) Efficacy of plant-produced recombinant antibodies against HCG. Hum Reprod 17: 2054-2061.

64. Bardor M, Loutelier-Bourhis C, Paccalet T, Cosette P, Fitchette A, et al. (2003) Monoclonal C5-1 antibody produced in transgenic alfalfa plants exhibits a N-glycosylation that is homogeneous and suitable for glyco-engineering into human-compatible structures. Plant Biotechnol J 1: 451-462.

65. Putalun W, Taura F, Qing W, Matsushita H, Tanaka H, et al. (2003) Anti-solasodine glycoside single-chain Fv antibody stimulates biosynthesis of solasodine glycoside in plants. Plant Cell Rep 22: 344-349.

66. Rodríguez M, Ramírez N, Ayala M, Freyre F, Pérez L, et al. (2004) Transient Expression in Tobacco Leaves of an Aglycosylated Recombinant Antibody Against the Epidermal Growth Factor Receptor. Biotechnol Bioeng 89: 188-194.

67. Negrouk V, Eisner G, Lee H, Han K, Taylor D, et al. (2005) Highly efficient transient expression of functional recombinant antibodies in lettuce. Plant Science 169: 433-438.

68. Girard LS, Fabis MJ, Bastin M, Courtois D, Pétiard V, et al. (2006) Expression of a human anti-rabies virus monoclonal antibody in tobacco cell culture. Biochem Biophys Res Commun 345: 602-607.

69. Brereton HM, Chamberlain D, Yang R, Tea M, McNeil S, et al. (2007) Single chain antibody fragments for ocular use produced at high levels in a commercial wheat variety. J Biotechnol 129: 539-546.

70. Sunilkumar G, Waghela SD, Campbell LM, Rathore KS (2009) Expression of anti-K99 scFv in transgenic rice tissues and its functional characterization. Transgenic Res 18: 347-360.

71. Lai H, Engle M, Fuchs A, Keller T, Johnson S, et al. (2010) Monoclonal antibody produced in plants efficiently treats West Nile virus infection in mice. Proc Natl Acad Sci U S A 107: 2419-2424.

72. Lombardi R, Donini M, Villani ME, Brunetti P, Fujiyama K, et al. (2012) Production of different glycosylation variants of the tumour-targeting mAb H10 in Nicotiana benthamiana: influence on expression yield and antibody degradation. Transgenic Res 21: 1005-1021.

73. Winichayakul S, Pernthaner A, Scott R, Vlaming R, Roberts N (2009) Head-to-tail fusions of camelid antibodies can be expressed in planta and bind in rumen fluid. Biotechnol Appl Biochem 53: 111-122.

74. Giersberg M, Floss DM, Kirpiyanov S, Conrad U, Scheller J (2010) Covalent dimerization of camelidae anti-human TNF-alpha single domain antibodies by the constant kappa light chain domain improves neutralizing activity. Biotechnol Bioeng 106: 161-166.

75. Aviezer D, Brill-Almon E, Shaaltiel Y, Hashmueli S, Bartfeld D, et al. (2009) A plant-derived recombinant human glucocerebrosidase enzyme--a preclinical and phase I investigation. PLoS One 4: e4792.

Author Affiliations

Top

[1]*CONICET/ School of Pharmacy and Biochemistry, Universidad Maimónides, Hidalgo 775 (1404), 6th floor, Ciudad de Buenos Aires, Argentina*