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Review Article

Plant Derived Monoclonal Antibodies, Purification Techniques, and Economic Advantages

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Abstract

Monoclonal antibody products now account for approximately half of all pharmaceuticals coming to market. These medications are expensive to manufacture but are required to treat many diseases such as cancer, autoimmune diseases, and asthma. The technology now exists to manufacture these innovative drugs in plants. This process has been shown to significantly decrease the cost of these drugs and may potentially help reduce the economic burden for patients in need. This paper will go through the traditional and current manufacturing technologies for monoclonal antibodies and demonstrate economic advantages when they are produced in plants.

Keywords: *Agrobacterium*; Antibody Purification; B Cell Immortalization; Cell Transformation; Chinese Hamster Ovary Cells; Direct Gene Transfer; Hybridoma; Indirect Gene Transfer; Monoclonal Antibodies

Abbreviations

CHO: Chinese Hamster Ovary; dT: Deoxy-Thymidine; EBV: Epstein Barr Virus; HGPRT: Hypoxanthine Guanine Phosphoribosyl Transferase; ori: Origin of Replication; scFv: Single Chain Variable Fragment; SCW: Silicon Carbide Whiskers; T-DNA: Transferred DNA; TSP: Total Soluble Proteins; vir: Virulence Genes

Introduction

Monoclonal antibodies are an important part of cancer, autoimmune, and infectious disease therapy in the modern era [1]. Unfortunately, costs for cancer treatment with the use of monoclonal antibodies can be upwards of $35,000 per year [1]. Fabrication of monoclonal antibodies were first introduced in the 1970s and two main pathways for production existed at that time [2]. The first was the hybridoma technique introduced in 1975 by Kohler and Milstein, where the scientists were able to fuse spleen cells to myeloma cells in order to create a consistent line of antibody production [3]. The second method involved immortalization of B cells with antibodies of interest to become infected with the Epstein Barr virus [4]. This will create an established cell line as the B cells are transformed and characteristics preserved [4]. However, there are some problems with these two methods. Human monoclonal antibodies are hard to make, as there are not that many human myelomas to hybridize [2]. For B cell immortalization, you only have a small window of time to find an antigen specific B cell [4]. Also, only about 20% of B cells are transformed using the EBV (Epstein Barr virus) method [5].

Another option to create monoclonal antibodies is to fabricate them in plants [6]. Plant produced monoclonal antibodies provide

distinct advantages that include growing monoclonal antibodies on an agricultural scale [6], and absence of human or animal pathogens [7]. Furthermore, genetic material can be stored in seeds, provide cheaper products due to the large scale of growth, and multimeric forms of antibodies can be made from cross fertilization [6]. When comparing cost of goods from CHO (Chinese hamster ovary) cells, transgenic goat, and transgenic corn, we see that products from transgenic corn is the most cost effective [1]. Plants provide an alternative solution to consider when manufacturing monoclonal antibodies [1].

Market size and the economics of monoclonal antibody production

It was around the late 1990s when the first chimeric monoclonal antibodies were approved [8]. Since then, humanized and then fully humanized monoclonal antibodies entered the market place at an increasing rate [8]. From 2001 to 2002, the monoclonal antibody market was worth around $5.4 billion dollars [9]. By 2013, the total sales for monoclonal antibodies was around $75 billion with monoclonal antibodies representing half of all biopharmaceutical sales [8]. In the year 2020 the world market for monoclonal antibodies was estimated to be around $125 billion [10]. Finally by the year 2025, the market revenue for monoclonal antibodies is estimated to be around $300 billion [11] (Figure 1). Shifting to production costs, commercial facilities for antibody production can range from $40 million to $650 million [1]. A study in London looked at multiproduct facility sizes by Amgen, Genentech, Biogen and other biotech companies and discovered their facilities can be as large as 500,000 ft 2 with bioreactors reaching 200,000 L [1]. When looking at the figure 2 table (Figure 2), we can extrapolate the cost of a 200,000L bioreactor to be around $600 million [1]. Furthermore, costs for producing monoclonal antibodies may have hidden expenses such as depreciation, insurance, equipment maintenance of the HVAC system, documentation, and other quality assurance processes [1]. If we were to consider purification costs for monoclonal antibodies, we see that Protein A affinity chromatography, the most popular method [12], is the most expensive step [1]. Protein A affinity chromatography alone can cost upwards from $8000 to $15000 per liter [13]. The pressure to reduce production costs is great, so when we do a comparison between mammalian cell versus plant cell products, we see that plant derived products are more cost effective [1] (Figure 3). A 2007 study showed that CHO (Chinese hamster ovary) cell products can cost between $300-$3000 per 100 kg/year [1]. However, in the same study, products from transgenic corn only costs $50 per 100 kg/year [1] (Figure 4). In another study comparing alfalfa grown IgG to mammalian hybridoma IgG in a 250m2 facility, we see that it only costs about $500 to 600/g for alfalfa products versus $5000/g for the hybridoma antibody product [14]. With regards to manufacturing costs of monoclonal antibodies, we know that plant derived antibodies are extremely cost effective, as they are cheap to produce, inexpensive to store, easy to scale up, and safer than animal products [14].

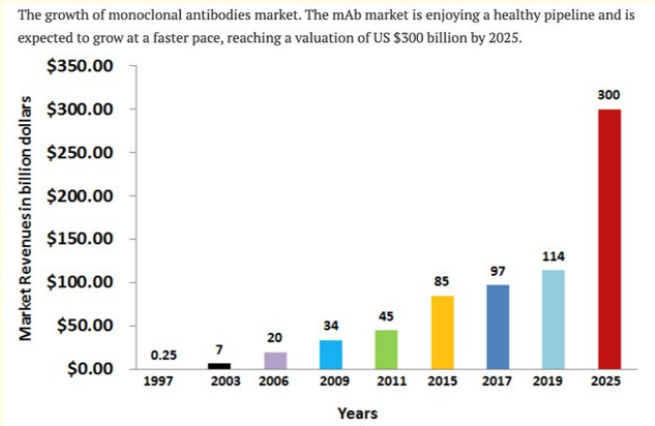

Figure 1: Bar graph illustrating the yearly growth of the monoclonal antibody market [11].

This graph shows the growth of the monoclonal antibody market up until 2025.

Capital investment costs for antibody facilities using mammalian cell culture

Manufacturing facility	Date facility completed	Capital investment (US $M)	Area (sq ft)	Production bioreactor capacity		
				Number	Size (L)	Total (L)
Genentech—Vacaville, CA, USA	2000	250	310000	8	12000	96000
Imclone—Branchburg BB36, NJ, USA	2001	53	80000	3	10000	30000
Biogen—LSM, RTP, NC, USA	2001	175	245000	6	15000	90000
Boehringer ingelheim expansion—Biberach, Germany	2003	315	–	6	15000	90000
Lonza biologics expansion—Portsmouth, NH, USA	2004	207	270000	3	20000	60000
Amgen—BioNext, West Greenwich, RI, USA	2005	500	500000	9	20000	180000
Genentech NIMO**—Oceanside, CA, USA	2005	380	470000	6	15000	90000
Imclone—Branchburg BB50, NJ, USA	2005	260	250000	9	11000	99000
Biogen Idec—Hillerød, Denmark	2007*	350	366000	6	15000	90000
Lonza biologics—Tuas, Singapore	2009*	250	–	4	20000	80000
Genentech expansion—Vacaville, CA, USA	2009*	600	380000	8	25000	200000

Figure 2: Table depicting the investment costs for mammalian cell culture antibody producing facilities from 2000-2009 [1].

This table shows a list of companies and their capital investments needed to operate mammalian cell antibody producing facilities.

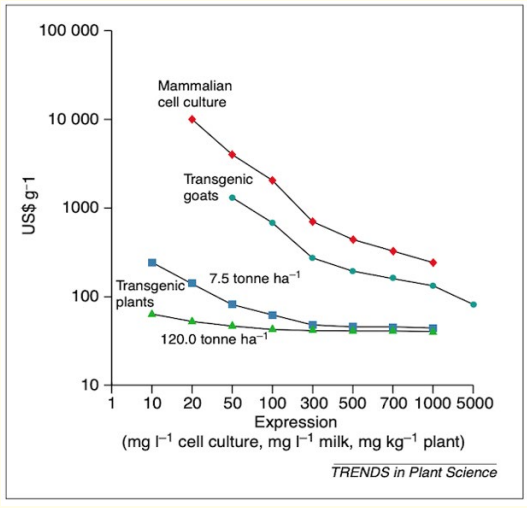

Figure 3: Graph depicting costs per gram to produce immunoglobulin A by different expression systems [14].

This graph highlights the lower cost of transgenic plants compared to other expression systems when producing immunoglobulin A. The represented costs include the cell culture and purification of immunoglobulin A. The blue line represents costs from seeds.

Comparison of cost per gram estimates for given production rates

Expression system	Cost of goods per gram ($/g)	
	100 kg/year	1000 kg/year
CHO cells	300–3000[a]	–
Transgenic goat	105[a]	36[a]
Transgenic corn	50[b]	14[b]

Figure 4: Table comparing cost per gram for different expression systems [1].

Transgenic corn is shown to be the most cost effect when compared to the other two expression systems in terms of cost of goods per gram.

a Source: Young., *et al*. M.W. Young, W.B. Okita, E.M. Brown, J.M. Curling, Biopharmaceutics 10(6) (1997) 34.

b Source: Mison and Curling D. Mison, J. Curling, Biopharmaceutics May (2000) 48.

Classical antibody production methods

Hybridoma monoclonal antibodies

This is an important process to cultivate new antibodies [15]. It starts by injecting an antigen of interest into an animal and then removing the B cells from its spleen [15]. However, because the B cells only survive for a short period of time, it is important to find a way to immortalize its existence [15]. The B cell can be merged to a myeloma cell by using either electroporation or polyethylene glycol [16]. This process creates a hybridoma that allows you to have a constant flow of antibodies of interest being produced [15]. We can isolate these new hybridomas from non-hybridoma cells, by putting them into a select media [15]. The special media will contain hypoxanthine guanine phosphoribosyl transferase (HGPRT), aminopterin, and thymidine kinase [2]. Cells go through two different types of pathways to make new DNA [15]. One is the de novo, which makes new DNA from precursors [2]. The other pathway, called the salvage pathway, involves recycling nucleosides and existing bases to make DNA [15]. The aminopterin blocks the de novo pathway, thereby forcing the cells to use the salvage pathway [15]. The salvage pathway requires the enzyme HGPRT [15]. Only the hybridomas and non-myeloma cells have this enzyme [15]. After a period of time only the hybridomas survive and now you can collect the monoclonal antibodies of interest [3] (Figure 5).

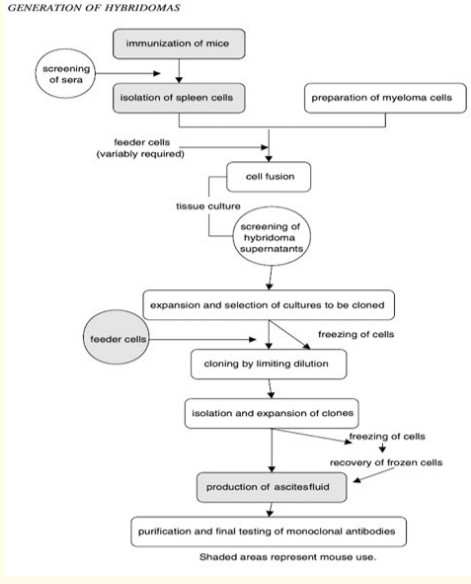

Figure 5: Schematic that shows the process of hybridoma antibody production [58].

This diagram describes the path of monoclonal antibody production. It starts with immunization of mice with the antigen of interest, creation of a hybridoma, and purification of the monoclonal antibody of interest.

B Cell immortalization using epstein-barr virus

B cell immortalization is another method to cultivate monoclonal antibodies [17]. We start this process by selecting individuals with immunization to novel antigens of interest [18]. When the Epstein-Barr virus infects a B cell, it transforms it to become a continuous growing antibody producing cell [19]. This happens because the Epstein -Barr virus nuclear antigen is now introduced into the transformed B cell [20]. Preselection of these antigen specific cells is a must before immortalization [17]. Once the EBV transforms the B cell, you can measure the existence of IgM, IgG, and IgA in the first 4-6 weeks [21]. The transformed B cells produce all classes of immunoglobulins, but IgM usually makes up the most in proportion [17]. A problem with B cell immortalization is that production of immunoglobulins tend to decrease over a period of time [17]. In fact, after 12-20 weeks of growth, specific antibodies become undetectable in the growth culture [17]. The transformed B cells need to be cloned before they stop making your antibodies of interest [17]. This can be done by limiting dilution [17] and growing them in agar [22]. Both of these methods require feeder cells that will support the growth of the transformed B cells [23]. These feeder cells can include non-dividing human fetal fibroblasts, umbilical cord blood mononuclear cells, and peripheral blood monocytes [17]. Researchers have also tried to pre-select antigen-specific lymphocytes to increase desired antibodies [18]. Several techniques have been tried, such as including antigen specific lymphocytes enriched by rosetting with antigen-coupled erythrocytes [22], fluorescence-labeled antigen bound to antigen specific cells and separated through fluorescence activated cell sorter [24]. Cells that did not bind to the antigen were removed [18]. Some researchers have argued against the use of EBV transformed B cell antibodies due to the relationship with human malignancies [25]. However, Crawford., *et al*. mentions that Epstein-Barr virus is ubiquitous and that most adults probably already have some form of it in their bodies [17].

Collecting gene of interest

Monoclonal antibody gene collection

If you do not have a library of existing useful monoclonal antibody genes, you can use the above-mentioned methods to collect the antibody gene of interest [2]. Once you have your monoclonal antibody of interest, you need to extract its mRNA from the target B cells, use a reverse transcriptase to convert it to cDNA, run it through a PCR, and then sequence the monoclonal antibody gene [26]. There are a few methods to capture mRNA which include reverse phase, ion exchange, size exclusion, hydrophobic interaction, and affinity [27]. According to Thermo Fisher, their most popular kit to purify mRNA is called Dynabeads mRNA Purification Kit [28]. This kit has oligo (dT) beads that will capture the poly A tail of mRNAs at the 3 prime end [28]. Ways of finding the monoclonal antibody DNA sequence in addition to the traditional Sanger sequencing, can be through the use of Edman degradation, de novo sequencing [29], and the mass spectrometer [30]. Once you have the DNA of the antibody of interest, you can now move on to transform plant cells to grow your monoclonal antibodies either through an indirect pathway such as using *Agrobacterium* or a direct pathway such as a gene gun or electroporation [31].

Indirect gene transfer

Agrobacterium transformation of plant cells

In 1988, It was shown that *Agrobacterium tumefaciens* was capable of transferring its T-DNA into an infected plant cell [32]. The process of transformation starts with a wounded plant that sends out phenols which attach to vir (Virulence) A receptors on *A. tumefaciens* [33]. This in turn activates vir G through phosphorylation [31], which causes production of various vir operon proteins which include vir D [33]. Vir D1 and vir D2 nick the transferred DNA (T-DNA) from the tumor inducing Ti plasmid on the left and right border and attaches to its 5' end. Vir B creates a pore between *A. tumefaciens* and the targeted plant cell [33]. The T-DNA is then inserted into the genome of the infected plant cell through this pore [33]. The T-DNA of *A. tumefaciens* contains all the enzymes to help *A. tumefaciens* grow [33]. These include auxins, cytokinin's, and opines [33] (Figure 6). However, if a gene of interest has to be inserted into the T-DNA for cell transformation, one must use either a co-integration or binary vector method prior to any monoclonal antibody production [31].

Co-Integration and T-DNA binary vectors

Because Ti plasmids and T-DNA regions often have restriction enzyme sites located elsewhere on the Ti plasmid, insertion of your gene of interest for plant cell transformation must be done in a specific fashion [34]. Therefore, before using *Agrobacterium* as a vehicle for plant cell transformation, one must figure out a method

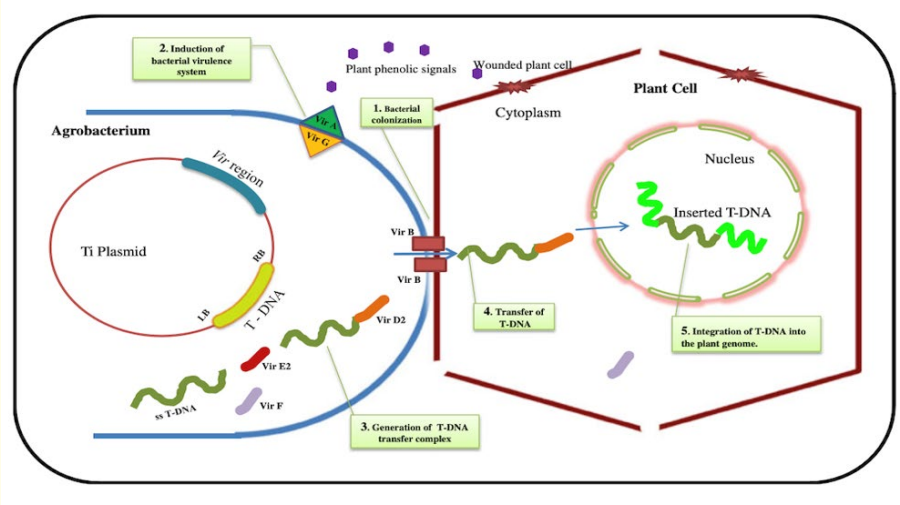

Figure 6: Diagram of plant cell transformation by Agrobacterium [33].

This diagram shows the process of plant cell transformation through Agrobacterium. The process starts with phenolic compounds secreted by an injured plant cell, which will activate the virulence A receptor. This in turn will activate virulence G. Virulence G will activate transcription of virulence D genes to make proteins that will detach T-DNA from Agrobacterium and send it to the plant cell through pores that are created by virulence B proteins.

to insert the antibody gene of interest into the T-DNA [34]. The first method of solving this problem is called co-integration strategy [35]. In this method, your gene of interest is first integrated on a *E.coli* plasmid that contains a sequence of the T-DNA [35]. Then this intermediate vector is integrated with a disarmed Ti plasmid, grown in *Agrobacterium*, through homologous recombination [35]. The disarmed (no oncogenes) Ti plasmid in the co-integrated process will have contained the vir genes, left and right borders of the T-DNA, and selectable markers [36] (Figure 7). The second and most commonly used method to clone your monoclonal antibody gene of interest into the Ti plasmid of the *Agrobacterium,* is the use of the T-DNA binary vectors method [34]. This method uses two plasmids [35]. The first one is the helper plasmid [37]. The helper plasmid, grown in *Agrobacterium*, would have the vir protein genes and an origin of replication (ori) site for *Agrobacterium* [37]. The second disarmed plasmid, grown in *E. coli,* would contain a multiple cloning site (MCS) for your gene of interest, a promoter site, origin of replication (ori) for both *E.coli* and for *Agrobacterium*, poly A, markers, and a reporter gene [37] (Figure 7). Both plasmids would end up in the *Agrobacterium* cell [37]. Either the T-DNA binary vector or co-integration method would be done first, prior to cell transformation of plant cells [37].

Direct gene transfer

Gene gun transformation

This method uses gold or tungsten coated with DNA and sent as a projectile into the cell of plants [38]. The DNA coated gold or tungsten is shot out of a gas acceleration tube using helium or an electric discharge [31]. The DNA to be transferred is within a vector that has an origin of replication, selective biomarker, a reporter gene like green fluorescent protein, and a promoter region [38]. It is also called biolistics and can deliver DNA to chloroplasts and mitochondria [39] (Figure 8). Once inside the cell, the DNA

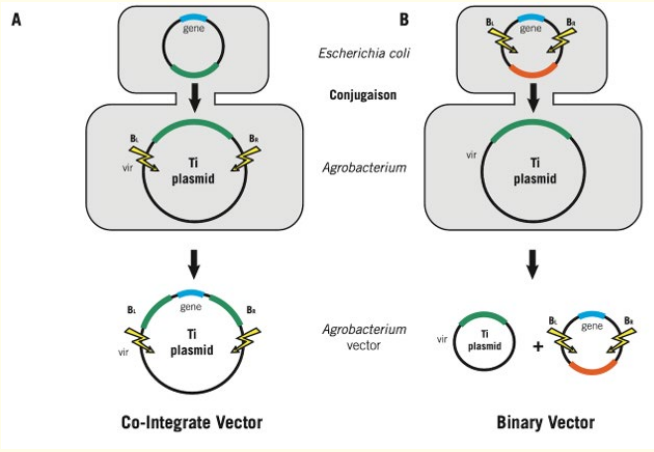

Figure 7: Comparison of co-integrate vector versus the binary vector methods of gene integration [36].

The co-integrate plasmid process starts by using an E. coli grown intermediate plasmid, which contains the vir genes, DNA of interest insert, selective marker such as kanamycin resistance, and gene sequences similar to the disarmed plasmid. The disarmed plasmid, grown in *Agrobacterium*, has the vir genes, a left and right border for gene sequences, and similar sequences with the intermediate plasmid to allow for homologous recombination. These two plasmids are then grown in a selective medium and then combined into one plasmid. The binary vector method uses a helper plasmid with the vir genes and a second disarmed plasmid that contains the DNA insert of interest, a selectable marker, and left and right border for gene sequences. The two plasmids would then be placed in the *Agrobacterium* cell for plant cell transformation.

can be transiently expressed or fully integrated into the plant cell genome [39]. Advantageous of the biolistics include a simple protocol, no need for a binary vector, and many types of cell types can be transformed [39]. Disadvantages of this method include high cost, messy integration patterns, and lacks control target to be transformed such as the nucleus, cytoplasm, or mitochondria [39]. Furthermore, it can only transfer DNA fragments smaller than 10kb, has a lower transformation rate, and is expensive [31]. Applications for this technology include gene silencing, plastid transformation, Mini chromosome delivery, and fluorescent dye delivery [38].

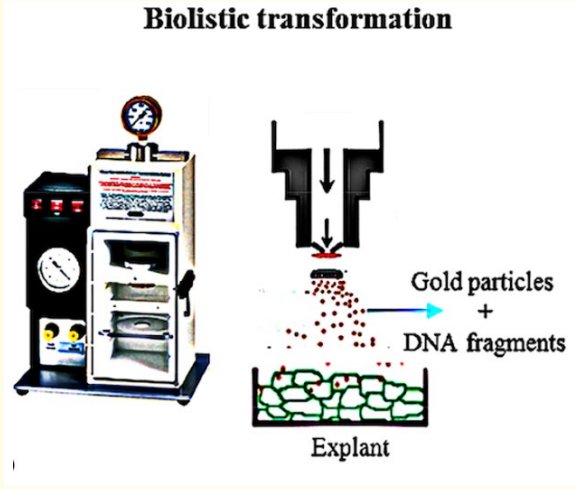

Figure 8: Gene gun diagram [59].

A gene gun will use pressurized helium to inject DNA coated gold or tungsten particles into plant cells for cell transformation.

Electroporation

Electroporation is another method to introduce DNA into the plant genome [31]. This method uses short, high field pulses of electricity to create pores in cell membranes [31]. In optimal conditions, the pores may re-seal themselves after the procedure is done [31]. However, because plant cells have thick cell walls, this method only works on a limited number of receptor species [40]. Another problem is that if the electric pulses are too strong, it may damage the DNA [41].

Liposome mediated transformation

This method involves the use of a phospholipid bilayer membrane spherical vesicle [42]. The phospholipid vesicle, called the liposome, carries the DNA of interest and can enter the target cell through endocytosis or membrane fusion [31]. The DNA and the liposomes are mixed together as the negatively charged DNA is attracted to the positively charged liposome [43]. When polyethylene glycol is added, this DNA liposome complex is able to enter the protoplast for the transformation process [42].

Microinjection mediated transformation

For this procedure, a glass microcapillary pipette injects a small amount of DNA solution into the cytoplasm or nucleus of

the plant cell under a microscope [44]. This method works well in mammalian cells, but may have some difficulty in plant cells due to the cell wall [42].

Silicon carbide whisker mediated transformation

Silicon carbide whiskers (SCW) are small needle like substances with sharp cutting edges that can cut into the cell walls and nucleus [45]. SCWs make pores into membranes of targeted cells which allows your DNA of interest to enter and start the transformation [42]. Furthermore, the DNA will not bind to the SWCs since the substance has a negative charge which is the same charge as DNA [42]. Unfortunately there is a carcinogenic risk when working with silicon carbide fibers and it also affects the regeneration of cells due to the damage during the transformation process [46].

Plant antibody purification

Once the monoclonal antibodies are created, it is time to purify them from the product solution [47]. The most useful methods for purification of plant antibodies with regards to production costs are immunoprecipitation and chromatography [47]. Affinity chromatography is the first choice for large volume processing as up to 90% of the monoclonal antibodies can be captured in the first step [12]. The protein A molecule has a histidyl residue that compliments the histidyl residue in IgG, which allows selectivity for that antibody [12]. Furthermore IgG is sensitive to low pH, which allows efficient elution of IgG from protein-A when the pH is 3 or 4 [12]. Protein G or protein A are very useful methods for monoclonal purification methods [47]. Protein A derives from *Staphylococcus aureus* that consists of a single polypeptide chain and can bind to the Fc region of IgG from various species such as monkeys, rabbits, mice, and humans [48]. Protein A can also bind to human IgM and IgA [48]. Protein G comes from Streptococcus and primarily binds to IgG from various species [49]. Large scale purification can also be done with a recombinant chromatography method [50]. Lastly, there is also protein L, which specifically binds to the light chains of the Fab regions [12]. Protein A and G bind to the fc regions [12]. Whether you use protein A, G, or L all depends on your target species or subtypes [12]. In order to isolate the exact antibody you are looking form you can use the antigen of interest that can be crosslinked to a support such as magnetic beads [51]. Then all the other antibodies with loose affinity can be washed away such that you now have your monoclonal antibody of interest [51].

Discussion

Plant derived antibodies have some advantageous over other methods of production [7]. These include the fact that plants can create single domain antibodies, Fab fragments, and single chain variable fragments [7]. We also know that bacteria cannot produce full sized antibodies and purified antibodies from mammalian cells can range from US $200- $2000 per gram [7]. However, there are some things to consider regarding plant antibody production. This can include differences in the chaperone proteins in the endoplasmic reticulum in mammalian cells versus plant cells [52]. Mammalian cells use Bip/GRP78 and GRP94 to direct folding and assemble heavy and light chains of antibodies [52]. Homologous chaperones have been found in plants, and scientists assumed not much difference in folding and processing occurs for mammalian antibodies in plants [52]. There are differences in total soluble protein (TSP) production between different plant species [53]. It was discovered that a particular antibody that had a 0.04% TSP in tobacco plants could have up to 3% of TSP in leaves of *Arabidopsis thaliana* [53]. Commercially successful biopharmaceuticals from plants have been few, which make companies reluctant to switch from traditional mammalian production [54]. Purification of proteins from CHO (Chinese hamster ovary) cells is rather straight forward, whereas purification of proteins from plants involves removal of large insoluble debris [54]. Plant biopharmaceutical products have lower yields when compared to mammalian cell products [54]. Finally, the patterns of glycosylation for proteins in plants differ from that of mammalian cells [55]. Glycosylation in plants contain (1,2)-xylose and (1,3)-fucose residues linked to N-acetylglucosamine [47] (Figure 9). These N-linked glycans may be immunogenic in mammals [56]. *In vitro* studies, these glycan differences did not appear to alter antigen binding affinity in mice [57]. Fortunately, researchers are working on humanizing the plant N-glycans [56]. One such group claims to have knocked out immunogenic plant xylose and fucose epitopes in *Arabidopsis thaliana,* allowing possible mammalian like antibody creation in plants [56].

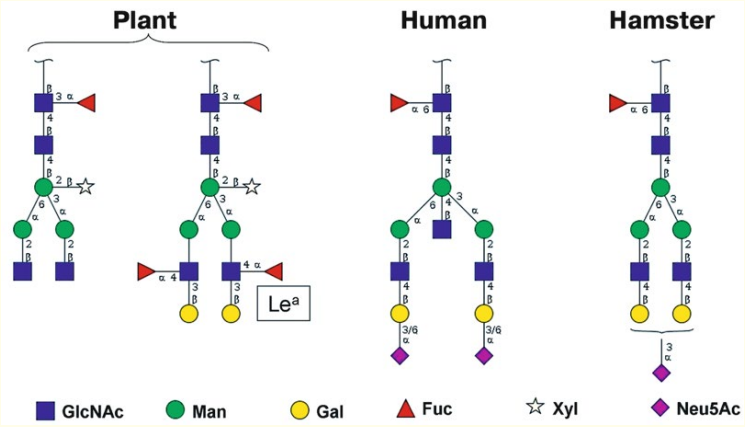

Figure 9: Glycosylation patterns between plants and mammals [60].

This diagram shows how plant cells have xylose attached to a mannose residue on the glycan core. In addition, plants have an α -1,3 fucose on the core of GlcNAc (N-Acetylglucosamine) as opposed to an α -1,6 fucose for mammalian cells.

Conclusion

Monoclonal antibodies have an upward trend in production and sales [8]. The cost of these treatments can be very expensive for the average consumer [1]. Creating monoclonal antibodies from plants may offer patients a more affordable treatment option [7]. From 2001 on, we have already seen four plant derived antibodies that may be useful in humans [14]. These include a chimeric IgG-IgA that works against *Streptococcus mutans*, the main agent for tooth decay, an anti-herpes virus antibody from soybean proved to be effective in a mouse model, an scFv (single chain fragment variable) for immunization against mouse lymphoma, and an antibody against carcinoembryonic antigen created in rice and wheat [14] (Figure 10). Despite some of the limitations of plant produced biopharmaceuticals, the startup costs for antibody production in plants is still much lower than that of mammalian cell cultures [14]. Because of this fact, considering plants as an alternative source of monoclonal antibody production should be continuously explored.

Application and specificity	Promoter	Signal sequences	Antibody name or type	Plant	Expression levels	Refs
Dental caries; streptococcal antigen I or II	CaMV 35S	Murine IgG signal peptides	Guy's 13 (SIgA)[a]	*Nicotiana tabacum*	500 μg/g FW[b] leaves	8,11
Diagnostic; anti-human IgG	CaMV 35S	Murine IgG signal peptides	C5-1 (IgG)	Alfalfa	1.0% TSP[c]	4
Cancer treatment; carcinoembryonic antigen	Maize ubiquitin	Murine IgG signal peptide; KDEL	ScFvT84.66 (ScFv)	Wheat	900.0 ng/g leaves; 1.5 μg/g seed	5
Cancer treatment; carcinoembryonic antigen	Maize ubiquitin	Murine IgG signal peptide; KDEL	ScFvT84.66 (ScFv)	Rice	29.0 μg/g leaves; 32.0 μg/g seed; 3.8 μg/g callus	5,6
Cancer treatment; carcinoembryonic antigen	Enhanced CaMV 35S	Murine IgG signal peptide; KDEL	ScFvT84.66 (ScFv)	Rice	27.0 μg/g leaves	5
Cancer treatment; carcinoembryonic antigen	Enhanced CaMV 35S	TMV Ω leader; murine IgG signal peptides; KDEL	T84.66 (IgG)	*Nicotiana tabaccum* (transiently with *Agrobacterium* infiltration)	1.0 μg/g leaves	13
B-cell lymphoma treatment; idiotype vaccine	TMV subgenomic coat protein promoter	Rice α-amylase	38C13 (scFv)	*Nicotiana benthamiana*	30.0 μg/g leaves	9
Colon cancer; surface antigen	TMV subgenomic promoter U5 CP	Murine IgG signal peptide; KDEL	CO17-1A (IgG)	*Nicotiana benthamiana*	Not reported	10
Herpes simplex virus 2	CaMV 35S	Tobacco extensin signal peptide	Anti-HSV-2 (IgG)	Soybean	Not reported	3

[a]SIgA, secretory IgA.
[b]FW, fresh weight.
[c]TSP, total soluble protein.

Figure 10: Table showing potential human therapeutics produced by plants [14].

Therapeutic and diagnostic plant made antibodies.

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Conflict of Interest

Authors declare that there is no conflict of interests.

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