Therapeutic antibody expression technology

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With the technological advances made during the past decade, antibodies now represent an important and growing class of biotherapeutics. With the potential new targets resulting from genomics and with methods now in place to make fully human antibodies, the potential of antibodies as valuable therapeutics in oncology, inflammation and cardiovascular disease can be fully realised. Systems to produce these antibodies as full-length molecules and as fragments include expression in both mammalian and bacterial cells grown in bioreactors and in transgenic organisms. Factors including molecular fidelity and the cost of goods are critical in evaluating expression systems. Mammalian cell culture and transgenic organisms show the greatest promise for the expression of full-length, recombinant human antibodies, and bacterial fermentation seems most favorable for the expression of antibody fragments.

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Abbreviations

cGMP	current good manufacturing practice
CHO	Chinese hamster ovary
GlcNAc	*N*-acetylglucosamine
scFv	single-chain Fv fragment

Introduction

Antibody therapeutics can potentially treat diseases ranging from autoimmune disorders to cancer and viral or bacterial infections. The number of monoclonal antibodies currently in development is higher than in any other therapeutic category, except for vaccines [1]. The emergence of antibodies as an attractive therapy is the result of the evolution of monoclonal antibody technology over the past 25 years from 100% mouse protein through chimeric and humanised proteins to fully human antibodies (Figure 1).

Recombinant expression technology in mammalian cell culture is the principal means for the commercial production of therapeutic antibodies; indeed, eight of the nine antibody products on the US market are recombinant molecules. These molecules are produced in mammalian cell culture using either Chinese hamster ovary (CHO) or mouse myeloma (NS0) cell lines. Although recombinant antibody fragments can also be produced in mammalian cell culture, other systems (e.g. bacterial fermentation) are preferred. Recently, antibody production in transgenic goats, chickens and various plant varieties has been developed to provide a high yield of product at a potentially lower cost of goods than that in mammalian cell culture. The major challenges to be overcome by these transgenic technologies involve the molecular fidelity of the expressed product (principally glycosylation) and the manufacturing regulatory issues raised by the Food and Drug Administration (FDA). This review will discuss the application of the different technologies for the production of therapeutic antibodies and antibody fragments. Focusing on progress made over the past year, our goal is to assess the value of each system with regard to molecular fidelity and cost of goods.

Evolution of antibody technologies

In 1975, Köhler and Milstein [2] established methods to make mouse monoclonal antibodies; however, the utility of these murine antibodies as human therapeutics was limited by their immunogenicity in humans [3,4••]. In the ensuing years, considerable effort was devoted to genetically engineering monoclonal antibodies in order to make them less immunogenic. The evolution of monoclonal antibodies from 1975 to the present day is shown in Figure 1. In 1984, chimeric monoclonal antibodies containing 66% human and 34% mouse sequences were engineered [5]. Approximately half of the therapeutic antibodies currently approved by the FDA are of this design. Between 1988 and 1991, techniques to successfully humanise murine antibodies using complementarity-determining region (CDR) grafting and veneering techniques were established [6,7], reducing the mouse proportion of the sequence to only 5–10%. An alternative approach for producing fully human antibodies is phage display [8]. The initial product is either a single-chain Fv fragment (scFv), in which a short polypeptide is used to directly link the variable heavy and variable light chains, or an Fab antibody fragment (Figure 2) from which a full-length antibody must then be constructed and produced using suitable expression systems [9]. The mid 1990s saw the emergence of transgenic mice that had been genetically engineered to contain a human antibody repertoire [10–12]. This technology allows the use of standard hybridoma techniques to generate fully human antibodies, obviating the need for humanisation altogether. The generation of fully human antibodies has now raised the standards for antibody manufacturing because the protein structure itself should not be immunogenic or have a short half-life.

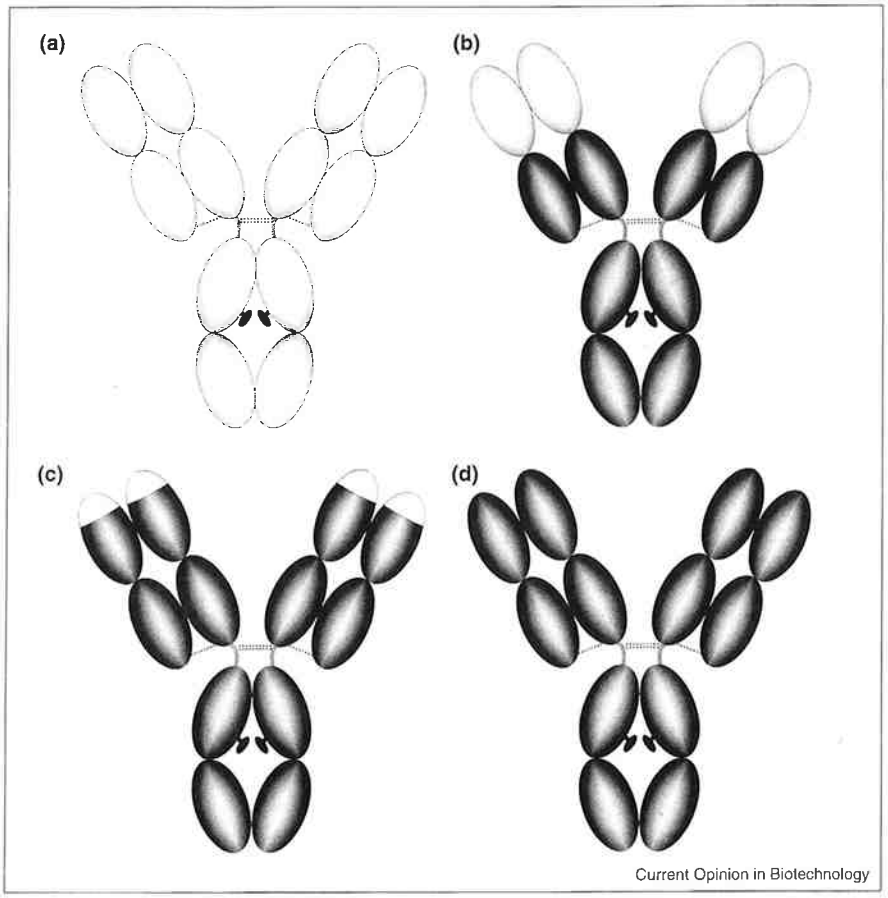
Factors to be considered during manufacture

In considering a suitable method for antibody manufacture a variety of factors must be evaluated. These include antibody structure, the importance of carbohydrate, and expression, which includes productivity, ease of purification and cost of goods.

Many different antibody structures have been generated using standard expression technology. These include full-length

Figure 1

Evolution of monoclonal antibodies from **(a)** mouse, through **(b)** chimeric (67% human), **(c)** humanized (90–95% human) and **(d)** human (100% human). Mouse-derived sequences (light shading) and human-derived sequences (dark shading) are shown.



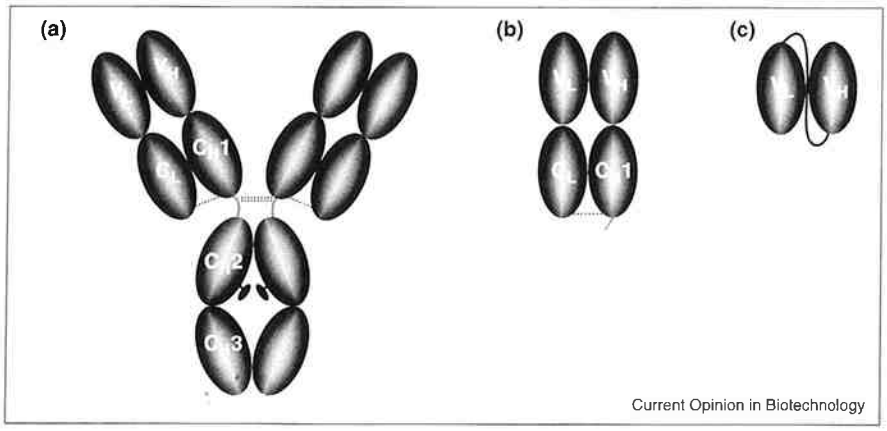
antibodies, antibody fragments (Fab or $[Fab']_2$), and scFv (Figure 2). If an antibody molecule of small size is preferred, for example, a fragment with no effector function or limited pharmacokinetic activity, then scFvs or Fab fragments generated in a bacterial system may be sufficient. ScFvs are highly selective for *in vivo* tumours, show good tumour penetration and reduced immunogenicity, and are cleared rapidly from the blood [13]. The use of these molecules is currently being directed towards radioimmunodetection and *in situ* radiotherapy (Table 1) [13,14]. If pharmacokinetic activity in the form of increased half-life is required for therapeutic purposes, however, then a full-length antibody is preferred. For immunoglobulin G (IgG), the molecule can be one of four subclasses: γ1, γ2, γ3 or γ4. If a full-length antibody with effector function is needed, a γ1 subclass is preferred. The γ1 and γ3 subclasses exhibit potent effector function, complement activation [15], and promote antibody-dependent cell-mediated cytotoxicity (ADCC) through interaction with specific Fc receptors [16,17].

Effector function in these subclasses requires the presence of an accessible carbohydrate structure at Asn297, within the Fc region of the antibody molecule. Unlike bacterial expression, animal cell culture and transgenic animal systems have the greatest potential to produce oligosaccharides similar to those contained in human antibodies. A comprehensive analysis has been conducted on 12 IgGs from different animals showing varying patterns of glycosylation [18••]. The complex biantennary structure of the *N*-linked oligosaccharide at Asn297 is constant across most animal species (Figure 3). Differences arise principally because of the presence or absence of fucose and bisecting *N*-acetylglucosamine (GlcNAc), and the presence or absence and type of sialic acid. Both human and chicken antibodies can contain oligosaccharides with only the sialic acid *N*-acetylneuraminic acid (NANA), whereas all other species can produce antibodies containing only the sialic acid *N*-glycosylneuraminic acid (NGNA) or a mixture of NGNA and NANA. Additionally, human, rat and chicken antibodies contain bisecting GlcNAc oligosaccharides. This structure is required for optimal γ1 effector function [19••]. Although CHO cells do not normally contain the enzyme activity (*N*-acetylglucosaminyltransferase-III) that leads to the bisecting GlcNAc moiety, a CHO cell can be selected or genetically engineered to display this activity [18••,19••].

Expression systems

There are various expression systems that can be used for the production of whole antibodies and antibody fragments. These include bacterial or mammalian cell culture

Figure 2



Structural summary of antibodies and antibody-derived proteins that are clinically useful. **(a)** A full-length antibody of the IgG subclass contains both heavy (V_H, C_H1, C_H2 and C_H3 domains) and light (V_L and C_L domains) chains. Interchain disulfide bonds are indicated (dotted lines). The single oligosaccharide chain (shaded knob) is attached to Asn297 within the C_H2 domain. **(b)** An Fab fragment contains V_H, V_L, C_L and C_H1 domains, along with a portion of the hinge region. Single-site pegylation can occur via a hinge thiol. **(c)** A scFv contains V_H and V_L domains connected via a polypeptide linker. Increased stability of scFv can be achieved by the introduction of an additional engineered disulfide bond. Fusion constructs of these molecules with nonimmunoglobulin domains have also been reported [37].

and transgenic animals or plants. As described above, the expression system of choice is partially dependent upon the intended use of the antibody, as well as the antibody yield derived from each system. This yield affects the cost of goods, which is comprised principally of two factors: the cost of the upstream process that generates the antibody, and the cost of the downstream process, that is, purification and final fill of the active pharmaceutical ingredient. For the sake of this review, only the cost associated with the upstream production process will be discussed.

Bacterial fermentation

Table 1 highlights the various expression systems used for the production of therapeutic antibodies. Bacterial systems are suitable for the expression of scFvs and Fabs (Figure 2). Because bacteria lack the cellular machinery to glycosylate proteins, only aglycosyl-antibody fragments are produced from bacterial fermentation. Recombinant fragments can be localised intracellularly or within the periplasm. The yield of scFvs in bacterial expression systems is usually low, primarily as a result of incorrectly folded protein retained either at the inner cell membrane or as an insoluble aggregate in the periplasm. Sánchez *et al.* [20] described the generation of 27–30 μg/ml of active scFv expressed in bacterial cytosol. Although the insoluble periplasmic protein can be extracted and refolded, high periplasmic expression can cause toxicity and cell death [20,21].

ScFvs have also been expressed in insect cells [13,20–24] (see Table 1). Functional scFvs have been secreted into the growth medium of a stably transformed *Drosophila* expression system at 25 μg/ml [23], and intracellularly in insect cells using the baculovirus expression system [23].

In contrast to bacterial and insect cells, there is some debate as to the ability of yeast cells to express functional antibodies; indeed, the glycosylation pattern in yeast is very different from that in mammalian systems [13,21].

Bacterial expression systems are cost-effective for the production of antibody fragments where no effector function or extended pharmacokinetic activity is required. To take economic advantage of bacterial expression, pegylation of the antibody fragment can be performed to increase the half-life of the antibody fragments in humans [25]. Because the assembly and glycosylation of full-length antibodies is not possible in bacterial systems, mammalian cell culture or transgenic organisms are better suited for production of these molecules.

Mammalian cell culture

Any human antibody IgG subclass can be generated in mammalian cell culture systems using either CHO cells, requiring methotrexate amplification, or NS0 cells, requiring methionine sulphoxime amplification (using glutamine synthetase selection in NS0 cells [26]). When expressing γ1 antibodies in a recombinant or transgenic system, the system must be chosen to maintain antibody effector function. An optimal effector function is dependent upon the correct carbohydrate structure at Asn297, including the presence of bisecting GlcNAc [19••] (see Figure 3). It must be appreciated that the structure and composition of oligosaccharides in immunoglobulins can vary considerably with the host cell or transgenic system chosen for expression.

In general, productivity in mammalian cell culture continues to increase because of improved recombinant expression vectors, the identification of 'hot integration spots' within the genome and the enhancement of biomass accumulation. This has resulted in cell-culture processes achieving yields of 1–2 g/L of unpurified antibody [27]. Further increases in the productivity of mammalian cell culture could be achieved by enhancing cell growth and

Table 1

Summary of antibody expression systems.

System	Antibody form	Source	Use	References
Bioreactor				
Escherichia coli	Mu scFv	Cytosol, periplasm	Diagnostic, therapeutic	[20–22]
Pichia pastoris	Mu scFv	Medium	Diagnostic, therapeutic	[13,21]
Drosophila melanogaster	Mu scFv	Medium	Diagnostic	[23]
Mammalian (CHO/NS0)	Ch IgG_1	Medium	Therapeutic	[38–41]
	Ch Fab			
	Hz IgG_1	Medium	Therapeutic	[42–44]
Hybridoma	Mu IgG_2	Medium	Therapeutic	[45,46]
	Mu IgM			
	Hu IgG_2	Medium	Therapeutic	[1,47]
Transgenic organism				
Animals				
Mouse	Ch IgG_1	Milk	Research	[28••]
Goat	Ch IgG_1	Milk	Therapeutic	[28••]
	Hu IgG_2			
Plants				
Tobacco	SIgA/G: Ch	Leaf	Therapeutic	[35,36]
Maize	Hz IgG	Seed	Therapeutic	[33]
Soy	Hz IgG	Pod, seed, stem, leaf	Therapeutic	[34]
Rice	Mu scFv	Seed	Diagnostic, therapeutic	[24]
Wheat	Mu scFv	Seed	Diagnostic, therapeutic	[24]

Ch, chimeric; Hu, human; Hz, humanized; Mu, murine; SIgA/G, secretory IgA/G.

the inherent specific productivity of the manufacturing cell line. Increasing the yield of antibody from a manufacturing process is particularly important where the product is to be used chronically and therapeutic doses are high. For some high-dose indications, >200 kg of purified bulk material may be required annually at a cost of goods of >$500/g. Assuming a productivity of 2 g/L with a 50% process yield, at least twenty 10 kL bioreactor runs per year would be required to meet this output. In order for the industry to maintain an acceptable margin on future production, the cost of goods must be reduced by 1–2 orders of magnitude — to tens of dollars per gram. The future cost of goods target for mammalian cell culture is below $50/g of final purified material.

Transgenic organisms

An alternative method for the large-scale production (e.g. hundreds of kilograms) of antibody is expression in transgenic animals or plants.

Goats

The generation of a transgenic goat herd able to produce antibody for phase I clinical trials takes up to 24 months. Antibody DNA fused to a milk-specific regulatory element is inserted into a single cell embryo by microinjection. Transmission of the mammary gland-specific transgene is achieved using Mendelian genetics [28••]. At this time, several monoclonal antibodies expressed in transgenic goat milk are in early-to-late stage development and clinical testing (Table 1). There is, as yet, limited clinical data on the efficacy and safety of antibodies generated in goat milk [28••]. Current figures suggest that, at 1000 kg/year, antibodies could be produced in goat milk for a cost of approximately $40/g [29], compared with mammalian cell culture in which the cost is $300–1000/g (at 100 kg/year assuming a titer of 0.5 g of antibody/L) [30]. The cost-effective advantage of transgenic animals is realised only at higher production requirements in excess of 75–100 kg/year; price model analyses that include both upstream and downstream process costs have concluded that, for only 10 kg of material in milk, the cost would be $300/g [30]. At this scale, mammalian cell culture is the more cost-effective option.

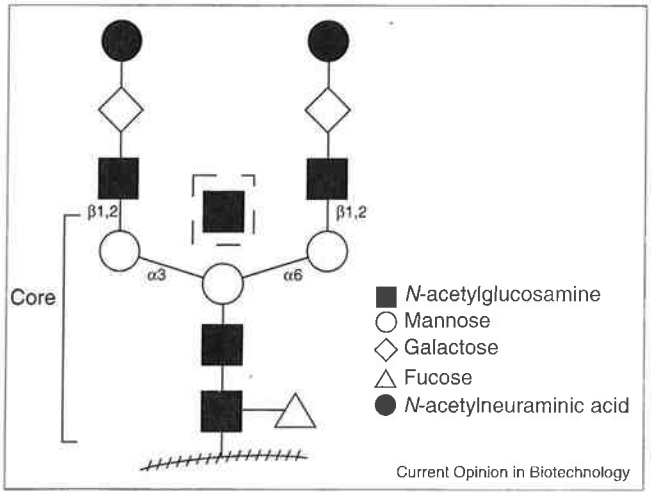
Chickens

The transgenic production of antibodies in egg white has been reported to take approximately 18 months. This expression system has the potential to supply large quantities of material for clinical trials relatively inexpensively [31]. For example, a flock of 5000 chickens is estimated to produce 125 kg of unpurified antibody/year (100 mg antibody/egg and 250 eggs/chicken/year). The commercial cost of chicken eggs (produced under conditions that do not comply with current good manufacturing practice [cGMP]) is currently $0.05/egg [31]. Hence, the cost of generating unpurified material from transgenic chickens is calculated at $0.5/g. Although this cost appears low, it does not account for the production of material under cGMP conditions. There are, as yet, no clinical data to support the production of therapeutic antibodies using this system.

Plants

The generation of transgenic plants for preclinical and phase I studies takes approximately 20 months. Antibody DNA is introduced into the plant using either

Figure 3



Representation of typical human Fc-associated oligosaccharide structures. A conserved oligosaccharide core, linked to asparagine, is composed of three mannose (Man) and two GlcNAc monosaccharide residues. Additional GlcNAcs are normally β1,2-linked to α6Man and α3Man, whereas the monosaccharide residues *N*-acetylneuraminic acid, galactose, fucose and the bisecting GlcNAc (boxed) can be present or absent, depending on the heterologous expression system that is used.

Agrobacterium infection or gene bombardment in the presence of a carrier molecule and gold particles. In the case of corn, antibody is produced in the seed; in tobacco plants, antibody is synthesised in the leaf. Recent advances with inducible promoters allow controlled expression only in harvested plant tissue so that antibody is generated in a GMP facility overnight and not out in the field [32•]. Although expression levels can be quite high, the carbohydrate structures generated in plants are quite distinct in composition and structure from human glycoproteins. To overcome this problem, aglycosyl-antibodies are typically produced in transgenic plants [33]. For IgG1 subclasses requiring effector function, this type of expression has obvious limitations; however, an aglycosyl mutein of IgG2 or IgG4, both of which lack significant effector function, might be successful.

The antibody huNR-LU-10 mAb, for example, generated in corn, has been genetically engineered to knockout the glycosylation site. The aglycosyl molecule was comparable in function to its glycosylated counterpart, although *in vitro* antibody-dependent cell cytotoxicity was reduced [31,33]. In addition, a humanised aglycosyl IgG1 to treat herpes simplex virus 2, produced in soybean, was compared with the glycosylated molecule produced in murine cells. Both antibodies were similar in affinity, neutralising activity and stability [34]. Also, a monoclonal IgA for the oral treatment of tooth decay, produced in transgenic tobacco, demonstrated higher functional activity and longer survival times than the murine IgG equivalent [31,33,35,36]. A rudimentary cost analysis conducted for plant-produced antibodies gave a figure of $100/g [30]. Growth in the field versus the greenhouse makes a difference in production cost. For field crops, production costs of only $43/g have been reported, whereas the generation of the same material in plants cultivated in greenhouses rises to $500–600/g [32•].

Conclusions

At this time, bacterial expression systems are only useful for the generation of antibody fragments. A full-length antibody molecule cannot yet be generated in this way; to make these, mammalian expression is the system of choice. Mammalian expression systems have been proven to generate safe and effective antibody molecules with serum half-lives equivalent to those observed for naturally occurring antibodies. Although there are differences in glycosylation patterns between antibodies generated in CHO versus NS0 cell lines, no clinical data suggest these structural differences have any effect on antibody activity *in vivo*. The majority of approved therapeutic antibodies are of subclass IgG1 and are generated in either NS0 or CHO expression systems. Both systems produce antibodies with acceptable effector function and serum half-life; moreover, human antihuman antibody (HAHA) responses owing to unusual carbohydrate structures have not been observed. Enhanced effector function can be obtained if an expression system is used that is able to generate antibodies featuring a bisecting GlcNAc structure. Transgenic production can achieve a lower cost of goods for large-scale manufacturing. Recent advances in the expression of antibodies in transgenic goats, chickens and plants — with respect to productivity, competitive timelines and cost of goods — suggest the need to seriously evaluate these recombinant systems as alternative platforms. If the productivity of mammalian cell culture continues to improve (e.g. to 2–5 g/L), so that the cost of goods can be reduced to tens of dollars per gram, then transgenics cease to be advantageous from a cost of goods standpoint, even for large-scale production. This assumes that there is no further reduction in the cost of goods for transgenic systems.

Comparing cost of goods analyses between cell culture and the various transgenic production systems, expression in transgenic chickens appears to show great promise when compared with goats and plants. With a growing number of antibodies moving into clinical evaluation, the utility of transgenic production will be evaluated alongside mammalian cell culture for the production of therapeutic antibodies.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Davis CG, Gallo ML, Corvalan JRF: **Transgenic mice as a source of fully human antibodies for the treatment of cancer.** *Cancer Metastasis Rev* 1999, **18**:421-425.

2. Köhler G, Milstein C: **Continuous cultures of fused cells secreting antibody of redefined specificity.** *Nature* 1975, **256**:495-497.

3. Richards J, Auger J, Peace D, Gale D, Michel J, Koons A, Haverty T, Zivin R: **Phase I evaluation of humanized OKT3: toxicity and immunomodulatory effects of hOKT3γ_4.** *Cancer Res* 1999, **59**:2096-2101.

4. •• Kipriyanov SM, Little M: **Generation of recombinant antibodies.** *Mol Biotechnol* 1999, **12**:173-201.

An excellent review that provides background information for all aspects of recombinant antibody engineering.

5. Morrison SL, Johnson MJ, Herzenberg LA, Oi VT: **Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains.** *Proc Natl Acad Sci USA* 1984, **81**:6851-6855.

6. Riechmann L, Clark M, Waldmann H, Winter G: **Reshaping human antibodies for therapy.** *Nature* 1988, **332**:323-327.

7. Padlan EA: **A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties.** *Mol Immunol* 1991, **28**:489-498.

8. Hufa GA, Hionjan I, Cuomo M, Koningsberger J, Wiegman L, Boel E, van der Vuurst de Vries A, Loyson S, Helfrich W, van Berge Henegouwen G *et al.*: **A recombinant, fully human monoclonal antibody with antitumor activity constructed from phage-displayed antibody fragments.** *Nat Biotechnol* 1999, **17**:276-281.

9. Groen L: **Antibody engineering via genetic engineering of the mouse: xenomouse strains are a vehicle for the facile generation of therapeutic human monoclonal antibodies.** *J Immunol Methods* 1999, **231**:11-23.

10. Groen L, Hardy M, Maynard-Currie C, Tsuda H, Louie D, Mendez M, Abderrahim H, Noguchi M, Smith D, Zeng Y: **Antigen-specific human monoclonal antibodies from mice engineered with human Ig heavy and light chain YACs.** *Nat Genet* 1994, **17**:13-21.

11. Mendez MJ, Green LL, Corvalan JRF, Jia X-C, Maynard-Currie CE, Yang X-D, Gallo ML, Louie DM, Lee DV, Erickson KL *et al.*: **Functional transplant of megabase human immunoglobulin loci recapitulates human antibody response in mice.** *Nat Genet* 1997, **15**:146-156.

12. Taylor LD, Carmack CE, Huszar D, Higgins KM, Mashayekh R, Sequar G, Schramm SR, Kuo C-C, O'Donnell SL, Kay RM *et al.*: **Human immunoglobulin transgenes undergo rearrangement, somatic mutation and class switching in mice that lack endogenous IgM.** *Int Immunol* 1994, **6**:579-591.

13. Freyre F, Vázquez JE, Ayala M, Canaán-Haden L, Bell H, Rodríguez I, González A, Cintado A, Gavilondo JV: **Very high expression of an anti-carcinoembryonic antigen single chain Fv antibody fragment in the yeast *Pichia pastoris*.** *J Biotechnol* 2000, **76**:157-163.

14. Ryu DDY, Nam D-H: **Recent progress in biomolecular engineering.** *Biotechnol Prog* 2000, **16**:2-16.

15. Brekke O, Michaelsen T, Sandlie I: **The structural requirements for complement activation by IgG: does it hinge on the hinge?** *Immunol Today* 1995, **16**:85-90.

16. Lund J, Winter G, Jones P, Pound J, Artymiuk P, Arata Y, Burton D, Royston J, Woof J: **Human FcγRII interact with distinct but overlapping sites on human IgG.** *J Immunol* 1991, **147**:2657-2662.

17. van de Winkel J, Anderson C: **Biology of human immunoglobulin G Fc receptors.** *J Leuk Biol* 1991, **45**:511-524.

18. •• Raju TS, Briggs J, Borge SM, Jones AJS: **Species-specific variation in glycosylation of IgG: evidence for the species-specific sialylation and branch-specific galactosylation and importance for engineering recombinant glycoprotein therapeutics.** *Glycobiolgy* 2000, **10**:477-486.

This paper describes the detailed analysis of the carbohydrate content of IgG molecules from 13 different species. It discusses the many different forms produced both within and between species.

19. •• Umana P, Jean-Mairet J, Moudry R, Amstutz H, Bailey JE: **Engineered glycoforms of an antineuroblastoma IgG1 with optimized antibody-dependent cellular cytotoxic activity.** *Nat Biotechnol* 1999, **17**:176-180.

This paper gives a good description of how engineering a bisecting GlcNAc into an IgG1 antibody resulted in increased antibody-dependent cell-mediated cytotoxicity activity.

20. Sánchez L, Ayala M, Freyre F, Pedroso I, Bell H, Falcón V, Gavilondo JV: **High cytoplasmic expression in *E. coli* purification and *in vitro* refolding of a single chain Fv antibody fragment against the hepatitis B surface antigen.** *J Biotechnol* 1999, **72**:13-20.

21. Cupit PM, Whyte JA, Porter AJ, Browne MJ, Holmes SD, Harris WJ, Cunningham C: **Cloning and expression of single chain antibody fragments in *Escherichia coli* and *Pichia pastoris*.** *Lett Appl Microbiol* 1999, **29**:273-277.

22. Grant SD, Porter AJ, Harris WJ: **Comparative sensitivity of immunoassays for haptens using monomeric and dimeric antibody fragments.** *J Agric Food Chem* 1999, **47**:340-345.

23. Reavy B, Ziegler A, Diplexcito J, Macintosh SM: **Expression of functional recombinant antibody molecules in insect cell expression systems.** *Protein Expr Purif* 2000, **18**:221-228.

24. Stöger E, Vaquero C, Torres E, Sack M, Nicholson L, Drossard J, Williams S, Keen D, Perrin Y, Christou P *et al.*: **Cereal crops as viable production and storage systems for pharmaceutical scFv antibodies.** *Plant Mol Biol* 2000, **42**:583-590.

25. Chapman A, Antoniw P, Spitali M, West S, Stephens S, King D: **Therapeutic antibody fragments with prolonged *in vivo* half-lives.** *Nat Biotechnol* 1999, **17**:780-783.

26. Bebbington CR, Renner G, Thomson S, King D, Abrams D, Yarrington GT: **High level expression of a recombinant antibody from myeloma cells using a glutamine synthetase gene as an amplifiable selectable marker.** *Biotechnology* 1992, **10**:169-175.

27. Zhou W, Chen C, Buckland B, Aunins J: **Fed-batch culture of recombinant NS0 myeloma cells with high monoclonal antibody production.** *Biotechnol Bioeng* 1997, **55**:783-792.

28. •• Pollock DP, Kutzko JP, Birck-Wilson E, Williams JL, Echelard Y, Meade HM: **Transgenic milk as a method for the production of recombinant antibodies.** *J Immunol Methods* 1999, **231**:147-157.

The authors give a clear discussion of the generation of transgenic goats. The expression of recombinant antibodies and methods to purify these molecules from goat's milk are discussed.

29. Fulton SP: **Transgenic production of human therapeutics.** In *Biopharmaceutical Process Economics and Optimization.* Washington DC: International Business Communications; 1999.

30. Russell DA: **Feasibility of antibody production in plants for human therapeutic use.** In *Current Topics in Microbiology and Immunology*, vol 240. Edited by Compans RW, Cooper M, Hogle JM, Kyoto YI, Koprowski H, Melchers F, Oldstone M, Olsnes S, Potter M, Saedler H *et al.*: Springer-Verlag; 1999:119-138.

31. Morrow KJJ: **Antibody-production technologies.** *Genet Eng News* 2000, **20**:1-55.

32. • Doran PM: **Foreign protein production in plant tissue cultures.** *Curr Opin Biotechnol* 2000, **11**:199-204.

This short review discusses in more detail the current status of protein expression in plants and compares the cost of goods for the production of recombinant β-glucuronidase between plants, goats and mammalian cell culture.

33. Baez J, Russell D, Craig J: **Corn seed production of therapeutic proteins moves forward. One company's experience.** *BioPharm* 2000, **13**:50-54.

34. Zeitlin L, Olmsted SS, Moench TR, Co MS, Martinell BJ, Paradkar VM, Russell DR, Queen C, Cone RA, Whaley KJ: **A humanized monoclonal antibody produced in transgenic plants for immunoprotection of the vagina against genital herpes.** *Nat Biotechnol* 1998, **16**:1361-1364.

35. Hansen E, Kawashima CG: **Delivery of immunoprophylactics in transgenic plants.** *BioDrugs* 2000, **15**:381-390.

36. Ma JK-C, Hikmat BY, Wycoff K, Vine ND, Chargelegue D, Yu L, Hein MB, Lehner T: **Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans.** *Nat Med* 1998, **4**:601-606.

37. Chamow S, Ashkenazi A (Eds): *Antibody Fusion Proteins.* New York: John Wiley & Sons; 1999.

38. Demidem A, Lam T, Alas S, Hariharan K, Hanna N, Bonavida B: **Chimeric anti-CD20 (IDEC-C2B8) monoclonal antibody sensitizes a B cell lymphoma cell line to cell killing by cytotoxic drugs.** *Cancer Chemother Radiopharm* 1997, **12**:177-186.

39. Lorber MI, Fastenau J, Wilson D, DiCesare J, Hall ML: **A prospective economical evaluation of basiliximab (Simulect) therapy following renal transplantation.** *Clin Transplant* 2000, **14**:479-485.

40. Knight DM, Trinh H, Le J, Siegel S, Shealy D, McConough M, Scallon B, Moore MA, Vilcek J, Daddona P *et al.*: **Construction and initial characterization of a mouse-human chimeric anti-TNF antibody.** *Mol Immunol* 1993, **30**:1443-1453.

41. Reverter JC, Beguin S, Kessels H, Kumar R, Hemmer HC, Coller BS: **Inhibition of platelet-mediated, tissue-factor-induced thrombin generation by the mouse/human chimeric 7E3 antibody; potential implications for the effect of c7E3 Fab treatment on acute thrombosis and 'clinical restenosis'.** *J Clin Invest* 1996, **98**:863-874.

42. Baselga J, Norton L, Albanell J, Kim Y-M, Mendelsohn J: **Recombinant humanized anti-HER2 antibody (Herceptin™) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/*neu* overexpressing human breast cancer xenografts.** *Cancer Res* 1998, **58**:2825-2831.

43. Johnson S, Oliver C, Prince GA, Hemming VG, Pfarr DS, Wang S, Dormitzer M, O'Grady J, Koenig S, Tamura JK, *et al.*: **Development of a humanized monoclonal antibody (MEDI-493) with potent *in vitro* and *in vivo* activity against respiratory syncytial virus.** *J Infect Dis* 1997, **176**:1215-1224.

44. Vincenti F, Kirkman R, Light S, Bumgardner G, Pescovitz M, Halloran P, Neylan J, Wilkinson A, Ekberg H, Gaston R, *et al.*: **Interleukin-2-receptor blockade with daclizumab to prevent acute rejection in renal transplantation. Daclizumab triple therapy group.** *New Engl J Med* 1998, **338**:161-165.

45. Smith SL: **Ten years of Orthoclone OKT3 (muromonab-CD3): a review.** *J Transplant Coord* 1996, **6**:109-119.

46. Blazar B, Deeg H, Heslop H: **Results of a Phase II multicenter study of ABX-CBL monoclonal antibody (anti-CD147) in patients with steroid resistant graft versus host disease (GVHD).** *Biol Blood Marrow Transplant* 2000, **6**:133.

47. Yang X-D, Corvalan JRF, Wang P, Roy C-N, Davis CG: **Fully human anti-interleukin-8 monoclonal antibodies: potential therapeutics for the treatment of inflammatory disease states.** *J Leuk Biol* 1999, **66**:401-410.