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Meeting the Challenges of IgG Expression: From Antibody Libraries to Clinical Supply

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A number of antibody drugs are currently in clinical development and 22 antibodies (including five diagnostic antibodies) have received FDA market approval in the last decade. A number of different technologies are now being used successfully to isolate potent therapeutic antibodies with minimal immunogenicity and improved safety. These include chimerisation (mouse/human antibodies), humanisation (complementarity-determining region [CDR] grafting), transgenic mice, phage display, ribosome display, and other emerging technologies.

The phage and ribosome display technologies used at Cambridge Antibody Technology (CAT) are based on the physical linkage of gene to gene product which enables the recovery and enrichment of genetic material encoding the selected antibody. Phage display libraries contain antibody variable region genes fused to a filamentous bacteriophage coat protein allowing the display of functional antibody fragments. Ribosome display libraries are made up of a complex of mRNA encoding the antibody variable region genes, ribosome, and the translated antibody fragment protein. In ribosome display, there is no stop codon at the end of the antibody-coding region, which prevents the release of the newly translated antibody fragment from the ribosome and maintains the gene product linkage.[1,2]

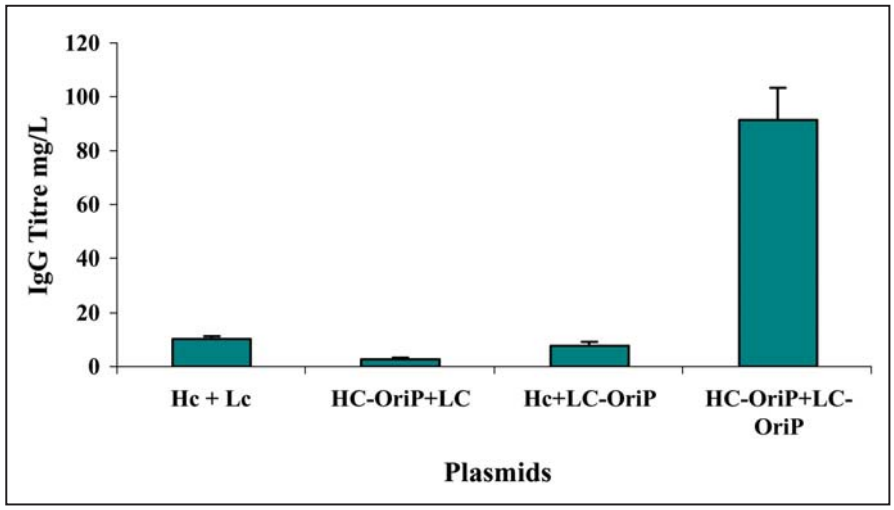

Figure 1. Comparison of human IgG expression from plasmids with and without OriP. Expression is enhanced when the OriP element is on both the heavy (Hc) and light chain (Lc) vectors.

Phage and ribosome libraries containing more than 10^{11} variants of antibody can be created. Employing different selection techniques, these libraries can be introduced to the antigen of interest resulting in the isolation of potent and highly specific antibodies including antagonist and agonist antibodies. For example, more than 1,000 distinct antibodies were isolated against just one antigen. High-affinity antibodies are often isolated directly from the library; however, when necessary, an additional process of affinity maturation can further improve affinity. Many highly potent antibodies, now at different stages of clinical development, have been isolated from the CAT libraries.[3-7]

After isolation of a highly potent and specific antibody, a key challenge is to ensure the capability for providing sufficient yield and quality to supply *in vitro* and *in vivo* proof-of-concept studies, as well as clinical trials. For both phage and ribosome display technologies, antibody fragments, usually single chain Fvs (scFvs), are isolated from the libraries. Generally, however, the preferred format for therapeutic antibodies is whole IgG because of the increased half-life, the ability to elicit effector

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functions compared to scFv, and perceived reduced immunogenicity.

This article highlights some of the challenges encountered and how they are addressed at CAT with respect to expression of IgG in quantities ranging from milligram amounts during the isolation of the lead candidate, to hundreds of grams during process development for clinical trial supply.

Different issues relating to IgG expression are confronted at different stages of drug discovery and clinical supply. Examples include speed of expression, expression titre, product quality, culture conditions, cell line stability, process scale-up, and regulatory issues. For the purposes of this article, the drug discovery stage refers to the identification and characterisation of a therapeutic antibody candidate while clinical supply stage refers to the cGMP production of the antibody candidate for clinical and market supply.

IgG Expression During Drug Discovery

During the early stages of drug discovery, antigen-specific *in vitro* potency assays are used to identify active scFv antibodies. Thereafter, a panel of potent scFv antibodies is selected for reformatting and further characterisation. Therapeutic candidates are chosen or further optimised based on results derived from whole IgG, partly because potency of the scFv is not always predictive of the added avidity component obtained from an IgG. Conversion to IgG is very successful, as about 90% of reformatted scFv show an increase in potency of, in some cases, up to 1,000-fold. In addition, *in vivo* proof-of-principle studies may also be required prior to commencing full cell line and process development for clinical trial supply. IgG requirements can therefore vary from 1–20 mg to aid in the selection of the lead candidate, to as much as gram amounts during the characterisation of the lead candidate. Consequently, different expression systems are used depending on the amount of IgG required for a particular assay or study.

A rapid IgG expression system is essential during the initial stages of drug development to facilitate prompt selection of the lead antibody candidate. As the initial requirements range between 1–20 mg of IgG, an ideal system should include a rapid reformatting vector system coupled with a fast, efficient, productive, and robust expression system. It should also be capable of producing high-quality IgG with the appropriate post-translational modifications.

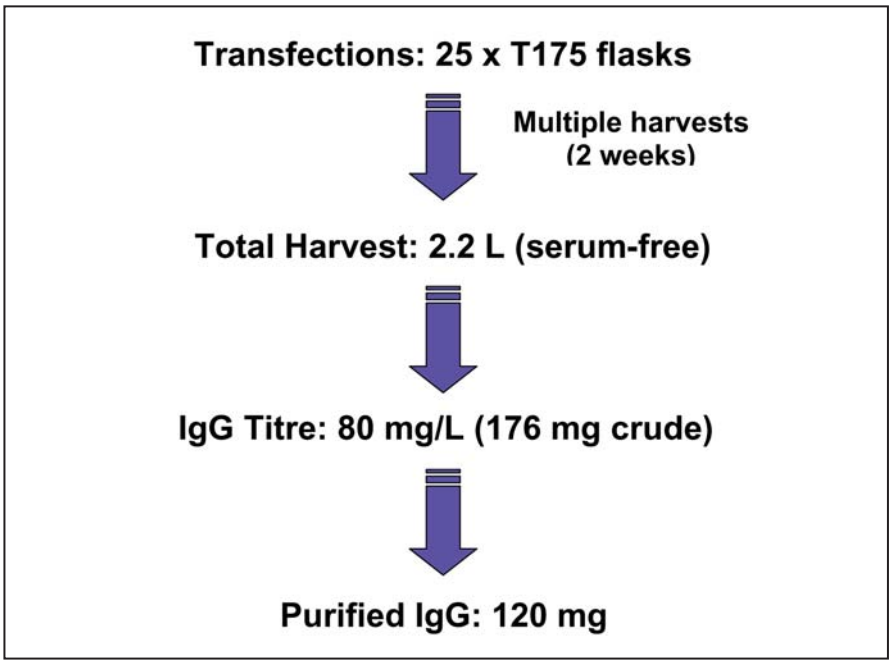

Figure 2. Large-scale transient expression system: overview of system and example of IgG titre and yield obtained. Over 100 mg IgG was purified from the above batch.

In order to reformat the scFv to the appropriate IgG isotype, rapid reformatting vectors have been developed which are amenable to high-throughput cloning. The cloning cassette uses a simple subcloning step that permits efficient reformatting of the variable heavy-chain and variable light-chain genes into the heavy- and light-chain expression vectors, respectively.[8]

Several rapid expression systems are used to produce milligram amounts of IgG. These include viral transduction of mammalian or insect cells and transfection of plasmid vectors for transient and stable expression. For transient expression, the following cell types are commonly used: human embryonic kidney cells (HEK 293), baby hamster kidney cells (BHK), Chinese hamster ovary cells (CHO), and COS cells.

Production of Up to 100 mg IgG

After extensive optimisation of the transient expression system (i.e., cell type, transfection method, expression vector, DNA concentration, and culture conditions), the HEK-293EBNA—OriP system is routinely used at CAT for rapid expression of up to 100 mg amounts of IgG. Different cell types, including HEK-293T, HEK-293EBNA, and COS cells were initially evaluated for transient expression of IgG. HEK-293EBNA cells were found to be superior, as confirmed by Durocher *et al.*[9]

HEK-293EBNA cells express the Epstein–Barr nuclear antigen-1 protein (EBNA-1). It is thought that the interaction of the Epstein–Barr virus OriP element with the EBNA-1 protein may promote nuclear import of plasmids carrying the OriP element following transient transfection as well as being responsible for episomal replication in primate cells. The expression vectors were therefore modified to include the OriP sequence. The expression is based on co-transfection of the heavy- and light-chain vectors into HEK-293EBNA cells. The expression-enhancing effect of OriP was found to be maximised when the element was present in both vectors (Figure 1).[10,11]

Further optimisation of the transfection method, DNA concentration, and media optimisations resulted in high

expression levels of crude IgG in serum-free medium (>200mg/L in some cases) within two weeks. This system is able to supply 100 mg of purified, active, and low-endotoxin IgG suitable for both *in vitro* and *in vivo* functional studies (Figure 2). This rapid expression system is extensively described in Prett *et al.* To improve the capacity of this system, a scalable suspension-culture transient system would be desirable. However, based on published data, the above reported expression titres may decrease when scaled up to a suspension system.[12,13]

Rapid Expression of More Than 200 mg of IgG

For later-stage development, more than 200 mg of IgG drug candidates may be required for further functional studies. Although the optimised transient expression method is potentially capable of providing this amount, it is very labour intensive and inefficient if subsequent batches are required. Therefore, CAT uses two different stable expression systems to provide up to gram amounts of IgG: 1) an in-house expression system, and 2) a licensed expression system.

The in-house system is rapid but less productive. This system is based on expression vectors described in Persic *et al*, which are co-transfected into murine myeloma NS0 host cells and selected with the antibiotic G418.[8]

Transfectants are isolated 10–14 days post-transfection and further selection of high-expressing cell lines may take up to eight weeks. Productivity can be up to 80 mg/L in suspension shake-flask culture and a further five-fold increase in titre can be obtained in fed-batch processes. The system is scalable and can produce gram amounts from 10–14 weeks post-transfection (Figure 3).

The licensed system is slower than the in-house system, but is usually more productive. Based on the proprietary glutamine synthetase (GS) system licensed from Lonza Biologics (Slough, UK), this system uses NS0 or CHO as the host cell line and the antibody genes are cloned into proprietary expression vectors from Lonza Biologics. The final expression vector is a single vector that contains both the light- and heavy-chain genes

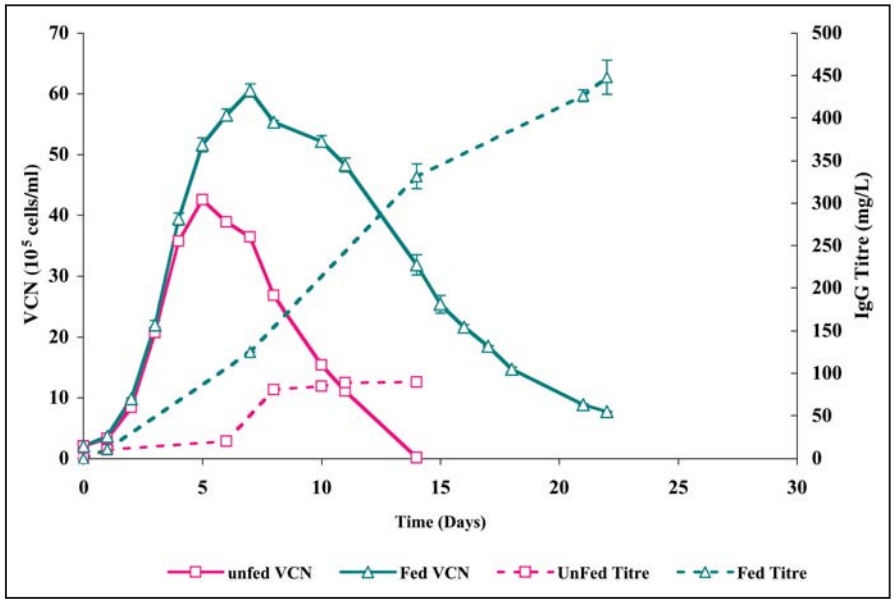

Figure 3. Graph showing the effect of feeding on a pEU-NS0 cell line expressing IgG. VCN is the viable cell number. Expression titre improved by up to five-fold in a fed-batch process.

and utilises the GS gene as a metabolic selective marker. NS0 cells are inherently GS(-) and CHO cells endogenously express GS. The addition of methionine sulphoximine (MSX; an inhibitor of GS) in glutamine-free media selects for CHO transfectants containing recombinant GS linked to the gene(s) of interest in either cell line. Transfectants are isolated after four or more weeks and then adapted to production medium before evaluating productivity. These cell lines can then be scaled up for large-scale production. Up to 100 grams have been generated within 18 weeks. Parental cell line productivity can range from less than 100 mg/L to more than 1g/L in shake-flask culture in unoptimised production medium. The use of optimised, serum-free, and chemically defined culture media, as well as feeding, has significantly enhanced productivity of GS-NS0 and GS-CHO cell lines by two- to three-fold at CAT.

Clinical Supply

IgG expression for clinical supply involves the development of productive cell lines, as well as fermentation and purification processes that need to be productive, robust at scale, and compliant with appropriate regulatory guidelines. This represents one of the most time-consuming stages during the manufacturing development of a drug. Among the different expression systems currently used to meet clinical and market supply are systems based on NS0, Sp2/0, CHO, and human cell lines. CAT has used GS-NS0 as it has a good track record and three products using this system have already received market approval.

Creation of a high-expressing, stable cell line usually involves vector construction and transfections followed by screening and selection of lead parental cell lines. Further work is then performed to ensure clonality and cell line stability. The process of establishing a robust process at scale can take up to 12 months.

Cell Line Cloning

Dilution cloning of parental lines is carried out to isolate a high expressing clonal cell line. Cloning ensures the consistency of product and may minimise cell line instability if the parental cell line is genotypically heterogeneous. A parental line with a highly heterogeneous population, in terms of productivity, usually generates unstable clones (in-house data). This is probably because the tendency to generate a heterogeneous population is inherent and cannot be abrogated by cloning.[14]

Subsequently, the clonal lines are adapted to the production medium and

a small panel of clones, ideally from different lineages, is chosen as leads for pre-seed stocks (PSS) of a cell line for cell banking. The panel is subjected to further characterisations such as cell line stability studies and process development (including media development). A final PSS with the appropriate growth and expression characteristics and expected adventitious agent profile is chosen for securing the master cell bank (MCB).

Cell Line Stability

Demonstration of stability in terms of cell growth, expression level, gene copy number, transcript identity, product quality, and identity during large-scale manufacture is essential before approval for market supply. These studies are carried out to confirm consistent production of the product of interest and suitability for the manufacturing process. Figure 4 shows the expression profiles of both a stable and an unstable cell line after a 60-cell-generation stability study of two lead GS-NS0 clones in unfed flasks.[14]

Based on data generated in-house after the molecular analysis of instability in GS-NS0 cell lines, the following observations were made: 1) instability is usually associated with a decrease in heavy-chain transcript and protein; 2) instability is associated with partial loss of heavy-chain gene; 3) non-IgG secreting population is predominantly made up of light-chain-only producers; 4) additional cloning does not eliminate the propensity for instability; and 5) propensity for instability may be associated with the target of the antibody expressed by the cell line. Barnes *et al* and Kearns *et al* both observed that instability in GS-NS0 was not linked to gene copy number. Barnes *et al* also observed a decrease in transcript level for both stable and unstable cell lines. They suggested that instability is probably due to RNA levels dropping below a critical threshold level in the unstable cell lines. Kearns *et al* concluded that the decrease in expression was due to a shift in cell metabolism, which was associated with cell age.[15-17]

Although still poorly understood, several mechanisms are likely to be responsible for cell line instability. However, based on data generated by Lonza Biologics, instability (with respect to consistency of product characteristics and suitability for manufacturing) occurs infrequently in GS-NS0 cell lines and many cell lines created are suitable for manufacturing.[18]

Suitability of a cell line for large-scale manufacture is strongly linked to its stability. It is therefore vital to screen out unstable lines early in the process and progress stable, robust, and productive cell lines into manufacturing.

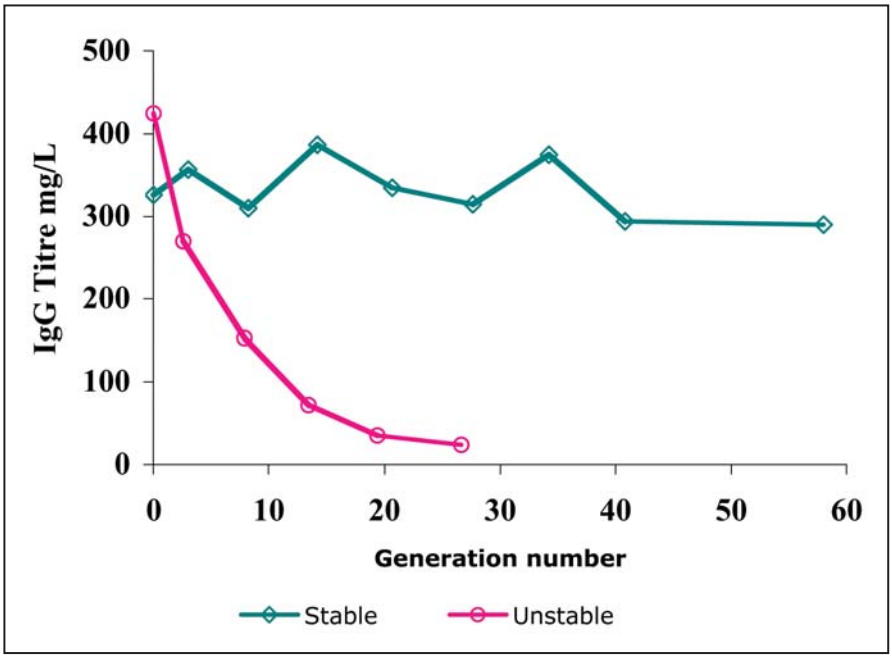

Figure 4. Final harvest titre from stable and unstable clones expressing human IgG.

Process Development

The challenges addressed during process development include development of a safe, growth-supporting, and expression-enhancing media that is suitable for operation in a large-scale bioreactor process, compliant with appropriate regulatory guidelines.

A glutamine-free, serum-free, and bovine serum albumin (BSA)-containing medium was developed to support antibody-secreting GS-NS0 cells. Human transferrin, which acts as an iron chelator, was also replaced with a safer, non-animal-origin system. Cells are adapted to the serum-free production medium as early as possible because expression levels in serum-containing medium are not predictive of expression levels in serum-free medium. This may be partly due to the increase in cell generation numbers (required to adapt cells to serum-free medium) exacerbating any cell line instability effects. Cells, therefore, require ranking based on growth and IgG titre in production medium before choosing the lead cell lines for process development.[19,20]

To further increase safety, compliance with regulatory guidelines, and efficiency, the GS-NS0 serum-free medium was developed into a chemically defined, protein-free medium. The lipid transport protein—animal-derived BSA—was replaced with commercially available synthetic lipid complexes and cofactors. The protein-free medium was optimised by augmenting trace elements. After successful optimisation of the fed-batch process for protein-free, chemically defined medium, its productivity now exceeds that of the protein-containing serum-free medium in a fed-batch bioreactor process (Figure 5). Productivity improvements observed with the chemically defined, protein-free, fed-batch bioreactor process have been reproduced with a number of NS0 cell lines expressing different antibodies.

Summary

A major challenge for all biopharmaceutical companies is to increase speed to clinic. Timelines can be short-

ened at different stages of antibody drug development. The advantage of phage and ribosome display is that the human antibody gene is linked to an antibody protein that can reduce the IgG reformatting time. The efficient transient expression system during the early programme stages, together with rapid IgG reformatting, contributes to the rapid identification of lead antibody drugs for further development.

The challenges of IgG expression encompass developing a rapid and efficient expression system during the early stages to facilitate rapid identification of lead candidates. During the early stages, expression level, cell line stability, clonality, and process development are of less importance because rapid, low-yielding expression systems are sufficient to generate high-quality IgG. In addition, there is less need for regulatory compliance at this early stage, except where the potency or safety results are intended to support a regulatory submission. However, for clinical and market supply, these factors are of critical importance in producing IgG in a way that is safe, robust, efficient, and economically viable. This inevitably affects the timelines.

Although different expression systems are used (as described above) for early stages and during clinical stages, differences in IgG activity and quality are rarely an issue, particularly if antibody effector function is not required for therapeutic rationale. This article describes the efficient IgG expression systems used at CAT that have enabled us to address the different challenges encountered, from choosing the lead drug candidate, to clinical supply. As these technologies evolve, biopharmaceutical companies will choose different and varying strategies to meet their needs for supply of IgG for proof-of-concept and clinical studies.

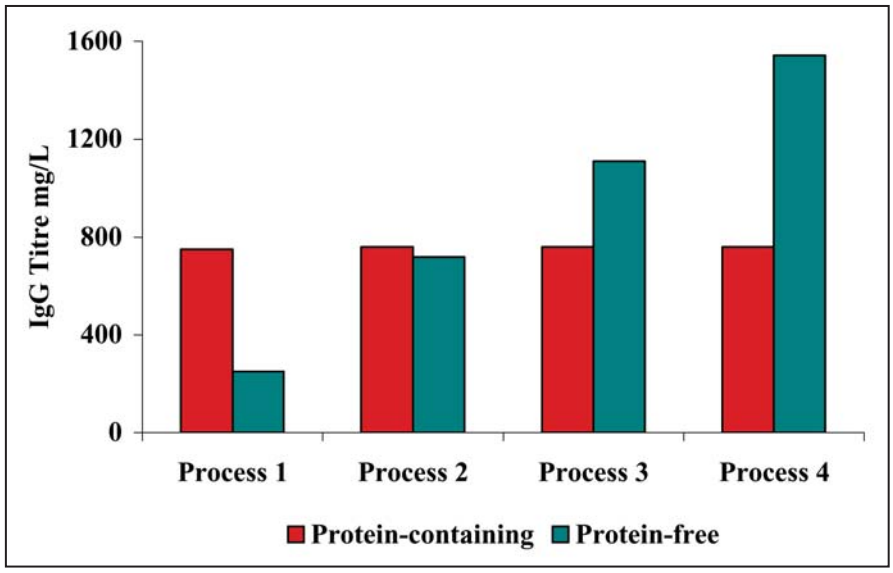

Figure 5. Graph showing the fed-batch IgG titre for protein-containing and protein-free processes. After extensive optimisation the protein-free process is now more productive than the protein-containing process.

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