Monoclonal antibodies (mAbs or moAbs) are monospecific antibodies which are made from identical immune cells that are all clones of a unique parent cell. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope [1]. Monoclonal antibodies (mAbs) are currently used for many diagnostic and therapeutic applications. Advances in the development of mAb-producing cell lines are being made, particularly regarding expression vector design and methods used for transfection, with the intent to create a reproducible methodology [10]. Selection of the most suitable clones is a critical step that can be improved, by including variables other than the expression level, which is still the common practice. Furthermore, strategies of cell engineering, although still mostly based on trial-and-error experimentation and not in standard protocols, hold great interest to improve cell growth and productivity, as well as product quality in the future. Improvements of the initial steps of the production process would not only result in cells with higher expression ability, but would also speed-up the process development. In this review article production, regulatory guidelines to cell engineering and applications of monoclonal antibodies are discussed.

Keywords: Monoclonal antibody, Hybridoma cell production, Human mAbs, Expression system, Expression vector, Cell engineering, Transfection methods, Vector design, Application of mAbs.

INTRODUCTION

Monoclonal antibodies (mAbs or moAbs) are monospecific antibodies which are made from identical immune cells that are all clones of a unique parent cell. Monoclonal antibodies have monovalent affinity, in that they bind to the same epitope [1]. Given almost any substance, it is possible to produce monoclonal antibodies that specifically bind to that substance; they can then serve as detector for purify that substance. This has become an important tool in biochemical, molecular biology and medicine [2]. Monoclonal antibodies (mAbs) are currently used for many diagnostic and therapeutic applications. The high demand for these biopharmaceuticals has led to the development of large-scale manufacturing processes, with productivity improvements and optimization of bioreactor systems. However, more recently, the early steps of production, prior to bioreactor culture, have been presented as alternative areas where productivity enhancements can be achieved. Thus, this review includes the progress made for the improvement of productivity in mammalian expression systems for the high production of mAbs. Advances in the development of mAb-producing cell lines are being made, particularly regarding expression vector design and methods used for transfection, with the intent to create a reproducible methodology [10]. Selection of the most suitable clones is also a critical step that can be improved, by including variables other than the expression level, which is still the common practice.

PURIFICATION OF MONOCLONAL ANTIBODIES

After obtaining either a sample of cultured hybridoma cells or a sample of ascites fluid, the desired antibodies must be extracted. The contaminants in the cell culture sample may consist of impurities primarily media components such as growth factors, hormones, and transferrins. In contrast, the in vivo sample is prone to have host antibodies, proteases, nucleic acids, and viruses. The sample is therefore condensed by ultrafiltration or dialysis. Most of the charged impurities are usually anions such as nucleic acids and endotoxins. These are often separated by ion exchange chromatography [3]. Either cation exchange chromatography is used at a low enough pH that the desired antibody binds to the column while anions flow through, or anion exchange chromatography is used at a high enough pH that the desired antibody flows through the column while anions bind to it [1].

The antibodies-containing media is then incubated with the immobilized antigens, either in batch or as the antibodies are passed through a column, where they selectively bind and can be retained while impurities are washed away. An elution with a low pH buffer or a gentler, high salt elution buffer is then used to recover purified antibodies from the support. To further select antibodies, the antibodies can be precipitated out using sodium sulphate or ammonium sulphate. The final purity can be analyzed using a chromatogram. Any impurities will produce peaks, and the volume under the peak indicates the amount of the impurity in the sample. Alternatively, gel electrophoresis and capillary electrophoresis can be carried out. Impurities will produce bands of varying intensity, depending on how much of the impurity is present [3].
Fig. 1: Schematic representation of production monoclonal and polyclonal antibodies [1,3,10,12]

Selected antigen (Immunization) → Rat

Remove spleen cells (Specific) → Cells fuse to make hybridoma

Hybridoma cells grow in selective culture medium

Individual hybridoma cells are cloned

Clones tested for desired antibody

Hybridoma injected in mouse → Remove ascitic fluid → Monoclonal antibodies purified

Selected clones are cultured and frozen → from culture supernate → Monoclonal antibodies purified

Application of antibodies to immunize animals against diseases

Bone marrow tumour + Culture myeloma cell (Immortal)
HUMAN MONOCLONAL ANTIBODIES

Ever since the discovery of monoclonal antibodies, the scientists have targeted efforts to avoid side effects of humanised and chimeric antibodies. Two promising approaches were identified — Phage display-generated antibodies and mice genetically engineered to produce human-like antibodies. One of the most successful commercial organisations behind the respective monoclonal antibodies was Cambridge Antibody Technology (CAT). Scientists at CAT demonstrated that phage display could be used such that variable antibody domains could be expressed on filamentous phage antibodies [4]. Monoclonal antibodies have been generated and approved for the treatment of disease like cancer, cardiovascular disease, inflammatory diseases, macular degeneration, transplant rejection, multiple sclerosis, and viral infection. In August 2006, the Pharmaceutical Research and Manufacturers of America reported that U.S. companies had 160 different monoclonal antibodies in clinical trials or awaiting approval by the Food and Drug Administration [7].

GUIDELINES TO CELL ENGINEERING FOR MONOCLONAL ANTIBODY PRODUCTION

The number of monoclonal antibodies (mAbs) presently used in clinical trials and already approved for therapeutic applications has increased significantly in recent years [9]. The production of mAbs in mammalian cells consists of a long process which involves steps of transfection of the genes of interest into the cells, selection of clones, adaptation to different culture conditions (usually suspension and serum-free medium), culture in bioreactors and scale-up to industrial level [10]. For this, the optimization of mAb production is usually performed in bioreactors, by testing different bioreactor, their modes of operation and culture parameters. In fact, it is known that several parameters such as cell line type, cell size and cell cycle, product characteristics, vector/promoter of transfection, methods of clone selection, post-transcription regulation [11] and growth medium, among many others, have great impact on the final productivity levels obtained. Therefore, the optimization of mAb production process should start in the first few steps of the process, and not only during bioreactor culture [10].

1. EXPRESSION SYSTEM

As already described, mammalian cells are currently the main hosts for commercial production of therapeutic proteins, including mAbs. Although a variety of either prokaryotic or eukaryotic (insect and plant cells) systems have been used, the fact that mammalian cells have the ability to perform correct (human-like), post-translational modifications makes them the most suitable system [13]. Production of mAbs in these cells necessarily begins with the development of a suitable cell line, determined by the selection of cell type. In order to be applied for biopharmaceutical application, cells must have the following characteristics:

(i) support high-level product expression over long periods of time maintaining high viable cell density and genetic stability,
(ii) be scalable,
(iii) have appropriate abilities for post-translational processing and (iv) allow appropriate characterization for human safety [12].

Furthermore, the choice of the best suitable cell line may also depend on the product application. If the aim is to produce small-scale quantities of mAbs, as for preliminary investigation (biochemical and biophysical) analysis, then African green monkey kidney (COS) cells may be appropriate. In fact, they have been used for transient expression of active antibodies since 1987. Actually, if the aim is large-scale production, the most currently used cells for this application are Chinese hamster ovary (CHO) cells, which have proved their importance both in laboratory and biopharmaceutical high-level mAb production [13].

Their suitability for large-scale and stable production of mAbs is due to their advantages of safety for use in humans, resemblance between glycan structure of their product with the natural human mAb, ease of transfection, presence of a powerful gene amplification system, ease of adaptation to growth in suspension and serum-free medium, and the ability to grow at high densities. Other cells commonly used for large-scale production are myeloma cells, such as SP 2/0, YB 2/0, NS0 and P3X63.Ag8.653 [9,14].

A variety of cell lines can be used for mAb production, although not all have the ability to achieve high-level of expression. However, the levels of expression appear to be more dependent on the combination of host cell, expression vector, transfection and selection strategy, rather than on only the characteristics of the host cell [12].

2. TRANSFECTION

After the selection of the most suitable cell type (for the application intended), cells are transfected to obtain mAb-producing clones. Transfection is the introduction of the product DNA into the cells and it involves different steps, from the design of the expression vectors and the transfection method (expression system and DNA delivery system) for the selection of the transfected cells with the most desirable characteristics [12,37].

2.1. EXPRESSION VECTOR

The expression of heterologous proteins in mammalian cells requires the use of specialized vectors to transfer the product gene into the cells [15].

These vectors should display three main features: 1. The expression levels should be independent from the site of integration in the genome,
2. The expression levels should be able to correlate with the number of integrated transgenic copies and
3. The expression efficiency should be maintained over time [16].

Due to these requirements, mammalian vectors are usually plasmids [15]. The ability to express genes in mammalian cells requires a cassette that consists of two types of elements:

1. Promoter/ enhancer elements that drive mRNA transcription,
2. Sequences that help to stabilize or enhance translation of the primary transcript [12].

Promoters are the elements that drive the expression of the recombinant gene, promoting and accurately positioning the beginning of transcription, while enhancers are elements that increase the level of transcription. For the expression of recombinant proteins in mammalian cells, a strong viral promoter/ enhancer or a cellular promoter/enhancer combination known to be particularly active to a certain host cell are mostly used [12].

Different elements have been used for stabilization and enhancement of translation of the primary transcript, including polyadenylation signals, Kozak sequence and intervening sequences. Polyadenylation signals derived from SV40 and bovine growth hormone gene are the most commonly used in mammalian expression cassettes and are thought to prolong the half-life of mRNA in cytoplasm and to enable efficient translation [17,37]. When the mammalian expression vectors are used for generation of a stable producing cell line, an extra sequence, encoding a selectable marker gene, is used. This selection gene can be present on the same vector as the recombinant gene or in separate vectors, and can be driven from a weak promoter, in order to increase the possibility to obtain high level of producer cells [17]. Furthermore, to improve the efficiency of stable transfection, plasmids can be linearized using restriction enzymes [15]. Depending on the surrounding chromatin at the integration site, expression of the product can be high, low or even null.

The development of synthetic promoters could solve many problems but their application would probably be cell-line specific [18]. Although a single vector system has the advantage of assuring equal introduction of genes into cells, the randomness of their integration into the cell genome remains a problem that results in inaccurate control of the relative expression of these genes [19].
2.2. TRANSFECTION METHODS

The introduction of the desired product gene into the host mammalian cell can be performed by a diversity of methods and systems, in a transient or stable way. The choice of method is again dependent on the application intended, as well as economic and technical factors [17].

2.2.1. TRANSIENT AND STABLE GENE TRANSFECTION

Usually the production of mAbs in mammalian cells for clinical or commercial purposes is done by stable transfection [17]. With the stable transfection technology, it is possible to obtain a continuous expression of the products by mammalian cells over prolonged periods of time. A stable transfection is accomplished by the integration of the DNA of the product gene into the genome of the host cell [12]. Although very similar, stable and transient transfection can be distinguished by the elimination of the steps of identification and selection of cells that have integrated the plasmid into the genome in transient transfection. This results in a faster process for obtaining MAb-producing cells [12]. Since the product DNA is maintained/replicated as an extrachromosomal unit, the expression ability is rapidly lost, only allowing the production of small (milligram to gram) quantities of mAbs [12, 18]. Therefore, transient expression systems are not suitable for large-scale production but are very useful for high throughput screening in drug discovery processes, in vivo evaluation and early product analysis [12, 19]. For transient systems, the efficiency of transfection is one of the most important factors, consisting in the percentage of cells taking-up and expressing DNA. On the other hand, in stable systems, the frequency of DNA integration into the chromosomes (copy number) and the position of integration become more important [12]. Nevertheless, for both systems, the expression levels are strongly affected by the strength of the promoter driving the expression of the product gene [12, 19].

2.2.1.1. GENE MARKER FOR STABLE TRANSFECTION

As mentioned above, for stable transfection, a marker gene is usually transfected with the genes of interest, conferring a selective advantage to the host cells [17]. The most commonly used marker genes are the dihydrofolate reductase (DHFR) and the glutamine synthetase (GS) [17, 19]. The DHFR expression system is commonly used with CHO cells and is based on the dhfr gene coding for the DHFR enzyme, which is involved in nucleotide metabolism, catalyzing the conversion of dihydrofolate to tetrahydrofolate. In this system, the selective advantage given to the transfected cells is the resistance to geneticin. Therefore, selection is performed by culturing the cells in medium which hypoxanthine and thymidine (H/T) and containing geneticin [17].

Furthermore, this system allows a process of gene amplification that ultimately increases the cell capacity of production, by the use of methotrexate (MTX), a drug that inhibits the DHFR enzyme [20].

Currently, there are more than 50 products in clinical trials [20] and two human therapeutic mAbs (Zanapax-Roche and Synagis (Medimmune) that use the GS system [17]. Apart from these two traditional gene markers, other expression systems for transfection have been developed, such as the OŞCARTM system from the University of Edinburgh. This system is based on a series of partially disabled minigene vectors that encode for hypoxanthine phosphoribosyltransferase (HPRT), essential for purine synthesis via the normal cellular salvage pathway. Apart from being quicker, this expression system has also been shown to achieve higher expression yields, with lower costs of goods, due to the absence of the specialized media and toxic chemicals needed for the more traditional systems. Moreover, the high yields of protein production that have been obtained with OŞCARTM have shown to be stable [21].

2.2.2. DNA DELIVERY SYSTEMS

For the introduction of the genes of interest into mammalian cells, several DNA delivery systems have been developed, with non-viral gene transfer approaches being the most suitable for manufacturing purposes [17]. These methods include calcium–phosphate precipitation, electroporation, lipofection and polymer-mediated gene transfer [17, 18].

2.2.2.1. CALCIUM–PHOSPHATE PRECIPITATION

This method was first described in 1973 by Graham et al., based on the formation of a fine DNA precipitate or complex that enters mammalian cells via an endocytic vesicle [22] and has the advantages of being inexpensive and working in a wide range of cell type [12]. Nevertheless, the still low efficiencies of this method, the requirement of serum in the medium during transfection [23], as well as the high variability of the transfection outcome depending on minor alterations in procedure (relative concentrations of reagents and DNA) or environment (pH, temperature) [12], has led researchers to find better gene transfer methods [23].

2.2.2.2. ELECTROPORATION

Electroporation is a simple and rapid method used for genes delivery into cells. This is accomplished by a pulsed electric field that disrupts the voltage gradient across the plasma membrane and creates reversible pores that allow the entry of DNA into the cell. Though it is less cell type specific than other transfection methods, parameters like peak voltage and fall time of the discharge waveform need to be optimized in each case. This method usually results in lower post-transfection cell viability. Consequently, this method is more commonly used in small assays that require rapid and low levels of production [12].

2.2.2.3. LIPOFECTION AND POLYFECTION

Lipofection and polyfection both are the most recent, and probably most simple, transfection methodologies for gene delivery in a diversity of cells. Lipofection consists of cationic lipid-mediated gene transfer into the cell, whereas cationic liposomes form a complex with the negatively charged DNA. This method can be performed in the presence or absence of serum, with little or no toxicity, as well as with attached and suspended cells.

Polyfection, for its turn, refers to the gene transfer mediated by cationic polymers such as polyethyleneimine, PEI [24] and dendrimers [12]. They interact with DNA forming a polyplex that protects DNA from degradation before reaching the cell nucleus. This method can be used for gene delivery in serum-free suspension cultures [24]. As with lipofection, the optimal protocol needs to be empirically determined for each cell culture [12]. It has been suggested that the method delivering the highest number of plasmids into cells is also the one generating cell lines with the highest specific productivity [23].

2.3. SELECTION

Cells transfected are subjected to a process of screening or selection that lasts from growth recovery after transfection, through single-cell cloning, amplification, suspension and serum-free adaptation, until final clone selection. After transfection, mAb-producing cells are selected by being subjected to specific culture conditions that only allow survival and growth of cell clones expressing the marker gene products [12]. These clones are transferred as single cells to a second cultivation vessel, where the cultures are expanded to produce clonal populations. Clones are then evaluated in terms of growth and product (mAb) titer, and the highest producers are selected for another round of cultivation and analysis, to confirm whether the levels of productivity are maintained [17]. The gene of interest expressing the antibody may be co-amplified with the selective marker genes, resulting in enhanced expression levels and mAb productivity [12]. However, other specific cell properties are also important for the production process and should be included in the screening, such as:

- the ability of a clone to grow in serum free medium,
- resistance to apoptosis,
- cell characteristics (i.e. cell size has been considered the major cellular determinant of productivity,
- production kinetics suitable for the type of process used,
- stability of product formation and
- A general robustness of the cell line under the stress and shear conditions found in bioreactors [25].
Since the site of insertion of the product gene in the cell genome is known to affect stability of production, studies on the exact site of insertion, for example, using fluorescent in situ hybridization (FISH) may be very useful to identify specific regions of the genome associated with stability of recombinant gene expression. Another approach used to accelerate clone selection involves the use of miniaturized bioreactors or shake flasks to simulate the standard production bioreactor conditions. After adaptation of cells to growth in suspension and serum-free medium, an enriched medium similar to the final production medium and a similar feeding regime can be applied. Thus, the clones more suitable for maintenance of high-producing levels in these bioreactor conditions can be selected [22].

3. CELL ENGINEERING

In recent years, continuous efforts have been made for the application of genetic engineering to improve the utility of mammalian host cells in recombinant protein production and monoclonal antibodies. These techniques are used in an attempt to modify specific features of the host cells in order to enhance the protein yield as well as the quality [12]. Post-translational protein processing and folding or enhancing the ability to grow in nutritionally defined media. To achieve this, strategies of anti-apoptosis, metabolic engineering, engineering cells for hypothermic growth, as well as engineering of molecular chaperones and post-translational processing have been approached [22,25].

3.1. ANTI-APOTOPSIS

Anti-apoptosis engineering has been one of the main areas of research in the field of cell line development for protein production [12]. Mammalian cells are sensitive to their environment, and under stressful situations, such as nutrient deprivation, growth factor withdrawal, oxygen limitations, toxic accumulations, osmolarity decreases and excessive shear-stress levels, programmed cell death (apoptosis) is induced. Therefore, it is of considerable value to be able to down-regulate or prevent apoptosis in culture in order to increase the density of viable cells by suppressing cell death, resulting in extended culture lifespan, and to increase the cellspecific productivity by maintaining the cellular activity. This will enable the maximization of the volumetric productivity of therapeutic proteins [25]. Different anti-apoptotic strategies have been studied. Among them, non-genetic approaches are the easiest to implement in existing cell culture processes. They rely on delaying the onset of apoptosis, by periodic nutrient feeding after determining the time at which nutrients are depleted, the use of galactose instead of glucose as a carbon source, or the use of nucleosides such as adenosine.

Another approach consists in the over expression of anti-apoptotic genes that mammalian cells possess. By increasing the expression or activity of one or more of anti-death proteins, it may be possible to enhance cell survival under stressful conditions. The anti-apoptotic genes already tested; with positive results in terms of both cell density and protein titer are Bcl-2, Bcl-xL, and 30Kc6, Aven, XIAP, CrmA and E1B-19 K [12,25]. Furthermore, anti-apoptotic chemicals have also been used, such as suramin, N-acetylcycteine (NAC) and silkworm hemolymph [26], offering protection against apoptosis under serum-free conditions [12].

3.2. METABOLIC ENGINEERING

Another focus of genetic manipulation has been metabolic engineering as a way to indirectly increase cell growth and volumetric production, through the inhibition of the accumulation of toxic metabolic by-products, such as lactate and ammonia [12]. Two approaches are commonly used:

- Improvement of the efficiency of central carbon (primary) metabolism and
- The reduction in lactate accumulation [12,15].

The first approach is usually based on redirection of cells into pathways using energy more efficiently, for example, over expressing pyruvate carboxylase which increases the flux of glucose into the tricarboxylic acid cycle. For the second approach, partial disruption of the lactate dehydrogenase A (LDHA) gene has been used, as well as the over expression of ura cycle enzymes, carbamoyl phosphate synthetase I and ornithine transcarbamoylase that reduce the accumulation of ammoniums in the culture medium [26].

3.3. ENGINEERING CELLS FOR HYPOTHERMIC GROWTH

Low temperature cultivation is a simple and very effective method of controlling cell proliferation. It was observed that mammalian cells growing between 27°C and 32°C have a low specific growth rate, but they maintain a high viability and reduced contamination by endogenous cell proteins. Also, low temperature cultivation has been associated with increases in specific productivity [27]. Genetic engineering strategies to improve volumetric recombinant protein production at low temperatures have been developed, yet with mixed and not so positive results. These strategies focus on alleviating the growth suppression at low temperature and include down-regulation of cold inducible RNA-binding protein (CIRP) with the intent to boost growth properties at hypothermia and adaptation of cells to low culture temperature [27,28]. Alternatively, a Biphasic process, where cells are first cultivated at 37°C in the growth phase for high growth rates followed by a temperature shift to low culture temperature in the production phase for high productivity may be used to increase the volumetric productivity [28]. There has been some interest in host cell engineering strategies to target the secretory pathway, in order to enhance mAb production [29]. The most common approach involves the over expression of ER chaperones, which has achieved varied results in mammalian cells [19,28]. Actually, it has been observed that the effects of molecular chaperones on protein production are dependent on several factors which include the expression system used, the target proteins and the chaperones concerned. For example, protein disulfide isomerase (PDI) has been used for chaperone engineering, with it’s over expression on protein-producing cell lines resulting in either enhanced, decreased or even unaffected productivities.

Indeed, the over expression of several chaperones, co-chaperones, holdases and/or foldases, concomitantly, in a functionally meaningful ratio to modulate the secretory machinery in a Global fashion might be a better strategy [29].

3.5. ENGINEERING OF POST-TRANSLATIONAL PROCESSING (GLYCOSYLATION)

While optimizing processes to achieve higher yields, it is critical to monitor product quality changes at every stage of development. Glycosylation variation, which can impact in vivo functions and stability, is one of the most sensitive quality-related attributes. Cell culture factors, host cell selection and protein specific features influence the glycosylation pathway, leading to the production of proteins with variable/suboptimal clarity or functionality. In an attempt to achieve a defined level of glycosylation on proteins produced in mammalian cell culture and to improve effector functions (such as antibody-dependent cell mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), recent studies have focused on glycosylation engineering with specific manipulation of oligosaccharide structures. This manipulation can be achieved by the over expression of appropriate glycosyltransferases, either by enhancing glycan quality or by increasing homogeneity of native structures, or by introducing non-host cell residues to specialize glycan quality and functionality [12,28].

Examples of the former include the over expression of galactosyltransferase (increase in the galactose levels), sialyltransferase (increase in the sialic acid levels) and N-acetylgalcosaminyltransferase III (increase the fraction of bisecting N-acetylgalosamine residues) [30,31]. For the second approach, examples include the introduction of sialic acid in an a-2, 6 linkages to glycoproteins synthesized by CHO and BHK cells that lack the specific sialyltransferase responsible for this transfer [27].

4. ADAPTATION TO NEW CULTURE CONDITIONS

The most common processes for large-scale production of biopharmaceutical products; including mAbs and they require suspended cells and also the use of serum-free medium. Therefore,
after obtaining mAb-producing cells, they need to be subjected to a process of adaptation to these new conditions, which, nevertheless, can impose some limitations to the development of the process of production [12].

4.1. SUSPENSION

Large-scale production of mAbs in bioreactors is usually performed with cells growing in suspension medium. This is because the surface/volume ratio in suspension cultures is much higher than in adherent cultures, allowing increased cell densities and productivities to be reached [12]. However, most mammalian cells naturally grow adherently and their growth in suspension demands a process of adaptation. This adaptation is not possible to all cell types, which limits the choice of the cell line and can be very time-consuming. Adaptation of cells that are grown adherently in serum-containing medium to growth in suspension is usually accomplished using the same medium but changing cells to spinner vessels [12,18], shake flasks or even roller bottles, with agitation of 50–80 rpm [12]. To guarantee a successful adaptation some parameters are critical such as: The initial inoculum density and viability.

Therefore, it is of extreme importance that the low proportion of survival cells is in sufficient number so that expansion does not take too long or becomes stalled. For this reason, the suspension culture should be initiated with a suitable high cell density. Furthermore, a strict monitoring should be maintained over the culture during the adaptation phase, with cell growth and viability evaluated frequently for several passages and productivity levels should also be assessed since they can change during adaptation [12]. To ease the passage from adherent to suspension culture, two approaches can be followed: media optimization (for example, altering cation concentration) [29] and the use of specially treated glass and plastic ware [12].

Also, since cells are subjected to constant mixing/agitation during suspension culture, shear-stress can be inflicted on cells. To minimize damage caused by agitation, shear protectants such as Pluronic F68 are commonly used. It should be mentioned that any additions to the culture have implications to downstream processing (purification) and, therefore, need to be thoroughly analyzed [12,20].

4.2. SERUM-FREE MEDIUM

Serum is an essential component of any culture medium supporting cell proliferation. However, serum has many disadvantages for the production of human therapeutics, such as:

- variable concentrations of components between batches which jeopardize the process consistency;
- burden put on downstream processing;
- high costs and
- Risk of transmission of animal diseases to humans [32].

Therefore, the use of serum-free (SF) or protein-free (PF) media is currently one of the main goals of commercial cell culture [12]. However, this requires the development of a specific medium for each cell line, since there is no universal SF medium [33]. Extensive efforts have been made in order to identify serum substituting supplements that fulfil the complex nutritional requirements of mammalian cells, which is usually done by time consuming methodologies [32,33]. These methods include the Ham’s approach (gradual reduction in the concentration of undefined supplements in the culture), the Sato’s approach (reconstruction of the extracellular environment involved in supporting cell growth in vivo), the top-down approach (taking of an existing serum-supplemented formulation for a similar cell line and selection of the constituents that are stimulatory for growth) and the bottom- up approach (selection of a basal medium, analysis of individual components for their effects on growth and combination of them to make a serum-free formulation) [12].

Statistical experimental design can be used as an efficient way of screening large numbers of medium supplements and identifying the most important [34], in a time-efficient way, accelerating the process of SF media development. Furthermore, in the development of SF media for mAb production, components important for production (and not just cell growth) should also be considered. For the adaptation to SF media, basically two approaches can be used:

- A direct approach, where serum is completely removed in a single step
- A gradual/sequential approach, where serum concentration in the medium is slowly reduced [12].

In both approaches, cell growth occurs slowly, with low viabilities obtained for several passages, demanding constant monitoring [12]. However, even in lower concentration, the selective pressure should be maintained so that the high-level gene expression is preserved [12,28]. Also, it is interesting to note that SF adaptation can even be advantageous in terms of mAb production, as reported in several works that indicate significantly increased mAb production kinetics in SF medium with respect to those done in serum-containing medium [35].

APPLICATIONS OF MONOCLONAL ANTIBODIES

DIAGNOSIS

Once monoclonal antibodies for a given substance have been produced, they can be used to detect the presence of that substance. The Western blot test and immune dot blot tests detect the protein on a membrane. They are also very useful in immunohistochemistry, which detect antigen in fixed tissue sections and immunofluorescence test, which detect the substance in a frozen tissue section or in live cells [5,36].

CANCER TREATMENT

The treatment for cancer involves monoclonal antibodies that bind only to cancer cell-specific antigens and induce immunological responses against the target cancer cells. Such mAb could also be modified for delivery of a toxin, radioisotope, cytokine or other active conjugate [5]; it is also possible to design bispecific antibodies that can bind with their Fab regions both to target antigens and to a conjugate or effector cells. In fact, every intact antibody can bind to cell receptors or other proteins with its Fc region [36,38]. mAbs approved by the FDA include: Bevacizumab, Cetuximab, Panitumumab and Trastuzumab [6].

AUTOIMMUNE DISEASES

Monoclonal antibodies used for autoimmune diseases include infliximab and adalimumab, which are effective in rheumatoid arthritis, Crohn’s disease and ulcerative Colitis by their ability to bind to and inhibit TNF-α [6]. Basiliximab and daclizumab inhibit IL-2 on activated T cells and thereby help prevent acute rejection of kidney transplants [6]. Omalizumab inhibits human immunoglobulin E (IgE) and is useful in moderate-to-severe allergic asthma.

FDA-APPROVED THERAPEUTIC ANTIBODIES [3,6,36,38]

<table>
<thead>
<tr>
<th>Product name</th>
<th>Specificity</th>
<th>Product type</th>
<th>Indication</th>
<th>Year</th>
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<td>Mouse</td>
<td>Transplant rejection</td>
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<td>Reopro</td>
<td>GpIIb/GpIIa</td>
<td>Chimeric Fab</td>
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<td>Rituxan</td>
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<tr>
<td>Synagis</td>
<td>RSV</td>
<td>Humanized</td>
<td>Respiratory virus syncytial</td>
<td>1998</td>
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<tr>
<td>Campath</td>
<td>CD52</td>
<td>Humanized</td>
<td>Chronic lymphatic leukemia</td>
<td>2001</td>
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Examples: Below are examples of clinically important monoclonal antibodies [6,37,38]

<table>
<thead>
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<th>Mechanism/Target</th>
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<td>rheumatoid arthritis</td>
<td>inhibits TNF-α</td>
<td>Chimeric</td>
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<td>Ulcerative Colitis</td>
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<td>Adalimumab</td>
<td>rheumatoid arthritis</td>
<td>inhibits TNF-α</td>
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<td>Ulcerative Colitis</td>
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<tr>
<td></td>
<td>Basiliximab</td>
<td>Acute rejection of kidney transplants</td>
<td>inhibits IL-2 on activated T cells</td>
<td>Chimeric</td>
</tr>
<tr>
<td></td>
<td>Dalizumab</td>
<td>Acute rejection of kidney transplants</td>
<td>inhibits IL-2 on activated T cells</td>
<td>Humanized</td>
</tr>
<tr>
<td></td>
<td>Omalizumab</td>
<td>moderate-to-severe allergic asthma</td>
<td>inhibits human immunoglobulin E (IgE)</td>
<td>Humanized</td>
</tr>
<tr>
<td>Anti-cancer</td>
<td>Gentuzumab</td>
<td>relapsed acute myeloid leukemia</td>
<td>targets myeloid cell surface antigen CD33 on leukemia cells</td>
<td>Humanized</td>
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<tr>
<td>Anti-cancer</td>
<td>Alemtuzumab</td>
<td>B cell leukemia</td>
<td>targets an antigen CD52 on T- and B-lymphocytes</td>
<td>Humanized</td>
</tr>
<tr>
<td></td>
<td>Rituximab</td>
<td>non-Hodgkin’s lymphoma</td>
<td>targets the HER2/neu (erbB2) receptor</td>
<td>Humanized</td>
</tr>
<tr>
<td></td>
<td>Trastuzumab</td>
<td>breast cancer with HER2/neu overexpression</td>
<td>targets the HER2/neu (erbB2) receptor</td>
<td>Humanized</td>
</tr>
<tr>
<td></td>
<td>Nivolumab</td>
<td>Approved in squamous cell carcinoma</td>
<td>EGFR inhibitor</td>
<td>Humanized</td>
</tr>
<tr>
<td></td>
<td>Cetuximab</td>
<td>Approved in squamous cell carcinoma, colorectal carcinoma</td>
<td>EGFR inhibitor</td>
<td>Humanized</td>
</tr>
<tr>
<td></td>
<td>Bevacizumab</td>
<td>Anti-angiogenic cancer therapy</td>
<td>inhibits VEGF</td>
<td>Humanized</td>
</tr>
<tr>
<td></td>
<td>Palivizumab</td>
<td>RSV infections in children</td>
<td>inhibits an RSV fusion (F) protein</td>
<td>Humanized</td>
</tr>
<tr>
<td></td>
<td>Abciximab</td>
<td>Prevent angioplasty in coronary angioplasty</td>
<td>inhibits the receptor GpIb/IIa on platelets</td>
<td>Humanized</td>
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</table>

CONCLUSION

The increasing importance of mAbs in therapeutic applications, occurring in recent years, has led to the rapid development of techniques/strategies for their large-scale production. Efforts continue on optimization of mAb production, but they usually focus on bioreactor design and/or operation. However, the procedures performed before bioreactor culture, such as transfection of the gene of interest into the cells, selection of the most appropriate clone and adaptation to different culture conditions, have been shown to strongly impact the expression levels possible to achieve in latter stages. Consequently, optimization should include and begin with these initial steps of creation of a mAb-producing cell line. Furthermore, these procedures are very time-consuming and, therefore, the ones most responsible for the delays observed until a mAb becomes commercially available. This is a major drawback for biopharmaceutical companies, whose success highly depends on how fast they can put their products in the market. Studies made in this area show that cell engineering, in particular, holds a great potential to surpass the obstacles currently encountered. Indeed, developments in this field may allow a better control over cell growth and productivity, enhance their ability to adapt to new conditions, as well as increase the quality of the product obtained. However, some results have been contradictory, which can be related to the lack of knowledge about cellular mechanisms and specifically their interconnections. If the current know-how is not expanded, success will strongly depend on trial-and-error experiments, and progress in this field will be dependent on new discoveries and their application.

REFERENCES

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