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Review

The history of monoclonal antibody development – Progress, remaining challenges and future innovations

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HIGHLIGHTS

- Monoclonal antibodies are the fastest growing group of pharmaceutical molecules.
- About 30 monoclonal antibodies are currently FDA-approved for clinical use.
- Research is now focused on their development and ways to maximize their efficacy.
- Issues surrounding their commercial viability have yet to be fully overcome.

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ABSTRACT

As medicine progresses into a new era of personalised therapy, the use of monoclonal antibodies to treat a wide range of diseases lies at the heart of this new forefront. Since the licencing of the first monoclonal antibody for clinical use 30 years ago, the monoclonal antibody industry has expanded exponentially and is now valued at billions of dollars.

With major advances in genetic sequencing and biomedical research, much research into monoclonal antibodies now focuses on identifying new targets for development and maximising their efficacy for use in clinical practice. However, a balance has to be struck with regards to reducing numbers of side-effects and overall economic cost, which arguably somewhat blighted their early clinical and commercial successes.

Nowadays, there are approximately 30 monoclonal antibodies that have been approved for use in clinical practice with many more currently being tested in clinical trials. Some of the current major limitations include: the use of inefficient models for generation, a lack of efficacy and issues of cost-effectiveness. Some of the current research focuses on ways to improve the efficacy of existing monoclonal antibodies through optimising their effects and the addition of beneficial modifications.

This review will focus on the history of monoclonal antibody development – how it has increasingly moved away from using laborious animal models to a more effective phage display system, some of the major drawbacks from a clinical and economical point of view and future innovations that are currently being researched to maximise their effectiveness for future clinical use.

1. Introduction

From the time the first monoclonal antibody was generated in 1975 and the first monoclonal antibody fully licenced in 1986, the field of monoclonal antibody development represents a novel way in which to target specific mutations and defects in protein structure and expression in a wide range of diseases and conditions. Today, with major rapid advancements in genetic sequencing and the translation of basic medical sciences research into clinical practice, humanised monoclonal antibodies are now the fastest growing group of biotechnology-derived molecules in clinical trials currently [1]. The global value of the antibody market is approximately $20 billion per year [2]. About 30 monoclonal antibodies are currently approved by the FDA for use in humans for treating various diseases and conditions including: cancer, chronic inflammatory diseases, transplantation, infectious diseases and cardiovascular diseases [3].

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2. Generation of monoclonal antibodies using the hybridoma technique

Monoclonal antibodies are monovalent antibodies which bind to the same epitope and are produced from a single B-lymphocyte clone [4]. They were first generated in mice in 1975 using a hybridoma technique [5]. The generation of hybridomas involves immunising a certain species against a specific epitope on an antigen and obtaining the B-lymphocytes from the spleen of the animal. The B-lymphocytes are then fused (by chemical- or virus-induced methods) with an immortal myeloma cell line lacking the hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) gene and not containing any other immunoglobulin-producing cells. These hybridoma cells are then cultured *in vitro* in selective medium (i.e. medium containing hypoxanthine-aminopterin-thymidine) where only the hybridomas (i.e. the fusion between the primary B-lymphocytes and myeloma cells) survive as they have inherited immortality from the myeloma cells and selective-resistance from the primary B-lymphocytes (as the myeloma cells lack HGPRT, they cannot synthesise nucleotides *de novo* as this is inhibited by aminopterin in the selective medium) [4]. The initial culture of hybridomas contains a mixture of antibodies derived from many different primary B-lymphocyte clones, each secreting its own individual specific antibody into the culture medium (i.e. the antibodies are still polyclonal). Each individual clone can be separated by dilution into different culture wells. The cell culture medium can then be screened from many hundreds of different wells for the specific antibody activity required and the desired B-lymphocytes grown from the positive wells and then recloned and retested for activity [6]. The positive hybridomas and monoclonal antibodies generated can then be stored away in liquid nitrogen.

3. Drawbacks of early monoclonal antibodies and possible developmental alternatives

The first licenced monoclonal antibody was Orthoclone OKT3 (muromonab-CD3) which was approved in 1986 for use in preventing kidney transplant rejection [7]. It is a monoclonal mouse IgG2a antibody whose cognate antigen is CD3. It works by binding to and blocking the effects of CD3 expressed on T-lymphocytes. However, its use was limited to acute cases due to reported side-effects (e.g. human anti-mouse antibody response) [8]. This is representative of the relative lack of early clinical and commercial success of monoclonal antibodies. A major stumbling block was the fact that the production of early monoclonal antibodies was limited by whether or not there was a suitable myeloma cell line available (usually mouse or rat). Hybridomas may also be low yielding or genetically unstable [6]. More recently, many different expression systems for monoclonal antibodies have been tested, each with contrasting effects. For example, *E. coli* was found to be an excellent system for expression of antibody fragments such as single-chain variable fragments (scFv) and antigen-binding fragments (Fab) [9]. However, the synthesis of a relatively larger, full-sized antibody (i.e. consisting of 2 heavy chains and 2 light chains joined together by disulphide bridges giving a total molecular weight of ~150 kDa) may be a step too far for such a relatively small microorganism, although the lack of glycosylating enzymes in *E. coli* may also prove to be beneficial for antibodies whose primary role is to block protein–protein interactions as opposed to invoking downstream immune effector responses (e.g. the complement system), which can lead to potential immunogenic side-effects [10]. Also, the transformation efficiency, and thus the purity of produced humanised monoclonal antibodies, has been found to be low during the use of transgenic animals [11]. This concept involves the use of animal species for the production of humanised antibodies. For example, endogenous mouse IgG genes can be deleted from transgenic mice and replaced with human copies of the genes. After immunisation, mouse B-lymphocytes synthesise human versions of the respective antibodies and hybridomas can be produced. Its advantages include: cognate pairing of variable heavy and light domains (VH/VL pairing), an *in vivo* antibody maturation process which generates higher affinity binding regions and full-length IgG antibodies produced without the need for further cloning [12]. Obtaining an easy source of monoclonal antibodies has also been explored through the expression of monoclonal antibodies in mammalian milk glands [13].

4. The generation of monoclonal antibodies using phage display

Another method of generating monoclonal antibodies is by using phage display [14]. This involves isolating B-lymphocytes from the blood of humans and then isolating the mRNA and converting it into cDNA using PCR to amplify all the VH and VL segments. These segments can then be cloned into a vector (usually as scFv) next to the PIII protein of a bacteriophage before being used to infect *E. coli* in order to generate a library containing approximately 10^{10} cells by inoculating the library with an additional helper phage [15]. *E. coli* can then secrete the bacteriophage containing the VH and VL segments as part of the bacteriophage coat. Specific VH and VL segments against the antigen can then be selected and used to reinoculate *E. coli* with the bacteriophage. Cells containing the plasmid can then be isolated and sequenced [16]. Its advantages include: once the library is made, the same library can be used to generate new antibodies and does not have to be remade, no immunisations are required as the entire process is done *in vitro*, antibodies can be obtained much more quickly than the traditional hybridoma technique and the library can be used to generate antibodies to toxic antigens that could not be used to immunise an animal [17].

5. Improvement of monoclonal antibody efficacy

Targets for improving antibody efficacy include: immunogenicity, antigen-binding affinity, effector functions and pharmacokinetics. Immunogenicity involves minimising non-human sequences by creating chimeric, humanised or human versions of the antibodies with as few T-lymphocyte epitopes as possible [18]. Antibody fragments are usually less immunogenic due to a lack of Fc domain [19]. Antigen-binding affinity can be improved by using phage display libraries to isolate antibodies with strong affinities for the antigen. However, sometimes antibodies with a lower affinity for the antigen may be required to allow better penetration of a tumour [20]. Effector functions can be improved by genetically engineering the Fc region to contain point mutations or glycan modifications. Yamane-Ohnuki and Satoh review and discuss the development of defucosylated antibodies which have increased affinities for the FcγRIIIa receptor and enhanced antibody-dependent cell-mediated cytotoxicity (ADCC) [21]. A particularly interesting aspect of antibody efficacy is its unique pharmacokinetic characteristics once inside the body. For example, it has been noted that the bioavailability of IgG in plasma is partially dependent on its interaction with the neonatal Fc/Brambell receptor (FcRn) [22]. The FcRn functions as a salvage receptor that leads to the rescue of IgG internalised in cells from degradation by lysosomes and causes recycling of antibodies into the plasma, thus prolonging its half-life [23]. The plasma half-life of IgG can also be increased by developing antibodies (e.g. through phage display) with increased affinity for FcRn [24]. Antibody fragments treated with polyethylene gycol (PEGylation) have also been shown to have an increased plasma half-life [25].

6. Beneficial modifications to monoclonal antibodies

Monoclonal antibodies can also be modified in order to have additional effects as well. Teicher and Chari discuss the possibilities of conjugated antibodies which involves coupling effector molecules to monoclonal antibodies (e.g. plant/bacterial toxins, enzymes, radionuclides, cytotoxic drugs, etc.) [26]. Chemical coupling of effector molecules to monoclonal antibodies is usually done with the aid of chemical ligands (i.e. joining molecules). Sites for coupling on the monoclonal antibodies usually include: thiol groups (e.g. –SH groups on cysteine residues, etc.), amine groups (e.g. $-NH_2$ groups on lysine residues) or carbohydrates. Cysteine, lysine or carbohydrate attachment sites can be added anywhere into a monoclonal antibody using site-directed mutagenesis, although the function of the monoclonal antibody must not be impaired [27]. For example, an antibody bound to a toxin can be targeted against a tumour. This can also be produced by genetic engineering where a DNA sequence coding for the specific toxin can be added to the end of the scFv chain region in the genome so they are transcribed at the same time [28]. Bispecific antibodies can target 2 separate epitopes with each arm of the Fab portion. They can be produced by chemical cross-linking of IgG, Fab or scFv fragments or through the generation of a 'hybrid hybridoma' [29].

7. Commercial issues surrounding monoclonal antibodies

Some of the relative lack of commercial success of monoclonal antibodies may be attributed to the high costs of its administration. For example, in leukaemia treatment, it costs approximately £37,000 for a year's supply of alemtuzumab [30]. Furthermore in cancer treatment, antibodies are rarely, if ever, curative. For example, bevacizumab (avastin) only extends median survival times by 30% (20.3 vs. 15.6 months compared with standard chemotherapy) [31]. Side-effects include first-infusion reactions (e.g. fever-like symptoms) [32]. Thus, issues surrounding the cost of administration, a need for greater clinical efficacy and side-effects still need to be addressed in order for monoclonal antibodies to become more commercially viable. Other factors that will influence the growth in commercialisation of monoclonal antibodies in the long-term include: quality control, patient compliance, a competitive pharmaceutical market and incentives for antibody development (e.g. infrastructure reimbursement programmes, etc.).

8. Conclusion

Monoclonal antibodies present an attractive option for the development of new therapies and molecular drug targets against a wide variety of common diseases due to their specificity and flexibility. Considerations when choosing the types of monoclonal antibodies to develop include: its method of production, avidity, its effector function and its delivery to its target tissue (e.g. a smaller scFv may be able to penetrate a tumour more effectively than a full-sized antibody). However, despite its drawbacks, there is still major interest from pharmaceutical companies to develop monoclonal antibodies for both clinical and diagnostic use and this will surely dictate the future of treatment and management of common, chronic conditions from a clinical and economical point of view.

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JKHL was involved in the study concept and design, data collection, data analysis and interpretation and writing of the paper.

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