CHAPTER I

INTRODUCTION

1.1 OVERVIEW

The treatment of cancer remains a formidable challenge owing to the difficulties in differentiating tumor from non-tumor cells in order to ameliorate the disease without causing intolerable toxicity to human patients. The use of monoclonal antibodies (mAbs) represents an attractive approach of overcoming this problem as the mAbs can be designed to selectively target tumor cells and elicit a variety of responses once bound. These mAbs can directly kill tumor cells by carrying toxic material to the target or can orchestrate the destruction of tumor cells in other ways, such as activating immune system components, blocking receptors or sequestering growth factors (Reichert & Valge-Archer, 2007).

Since mAbs could be produced continuously and with exquisite specificity using hybridoma technology (Kohler & Milstein, 1975), the economic promise of mAbs was expected to be limitless. Furthermore, mAbs have long biological half-lives in blood and tissues rendering them effective for prophylactic use, while the toxicity of infused mAbs were expected to be low due to their biological nature. Hence, mAbs were

referred as ‘magic bullets’ and enthusiastically hailed as the solution to cancer (Gavilondo & Larrick, 2000).

This was boosted by initial successful clinical outcomes of the treatment of lymphomas and leukemias using mouse mAbs (Levy & Miller, 1983) and in particular the approval of OKT3 anti-CD3 mouse mAb for acute renal transplant by Food and Drug Administration (FDA) of USA in 1986 (Shield et al. 1996).

However, when other mouse mAbs were used therapeutically, three factors that vastly limited their usefulness were identified. First, mouse mAbs administered in human elicited an anti-antibody response (AAR), referred to as human anti-mouse antibody (HAMA) response which developed in 50% of the treated patients (Khazaeli et al. 1994). Effector functions of mouse antibodies also proved to be less efficient in the human context and their biological half-life were also shorter when compared to human immunoglobulins.

In an effort to reduce the disadvantages and limitations of mouse mAbs, chimeric mAbs, consisting of mouse variable regions fused to human constant regions were designed (Morrison et al. 1984). Chimeric mAbs show the same specificity and affinity of parental mouse mAbs, and are capable of effector function in human context. Although chimeric mAbs do exhibit a longer biological half-life and possess less immunogenicity than mouse mAbs, nevertheless, chimeric mAbs can still elicit a reduced AAR, referred to as human anti-chimeric antibody (HACA) response (Presta, 2006).

Since the presence of mouse protein surface in chimeric mAbs are believed to be the reason for it being recognized by the human immune system, therefore humanized mAbs were designed whereby only the complementarity-determining region (CDR) of the mouse antibody were transplanted onto human variable frameworks (FWR) and human constant regions (Reichmann et al. 1988). The resulting CDR-grafted humanized mAbs had a reduction in immunogenicity compared to both of its mouse and chimeric mAbs counterparts. However, the loss of antigen-binding properties of humanized mAbs after CDR-grafting is also common, as a consequence of incompatibilities between mouse CDRs and human FWRs.

Hence, in this research, two alternative methods were utilized for the development of humanized anti-C2 mAbs (hum-C2 mAbs): a deimmunization method and a logical approach method. Both methods were respectively used to identify potential immunogenic mouse amino acids in the variable region of mouse anti-C2 mAbs which were then judiciously replaced with the corresponding residues from the highest homologous human sequences. The deimmunization method uses a computer algorithm and IgBLAST software, while the logical approach method uses only IgBLAST software. The functionality and immunogenicity of humanized anti-C2 mAbs developed using both methods were then compared.

Although there are various expression systems available for the production of recombinant proteins, including bacterial, yeast, plant and insect cells, mammalian cells continue to be the system of choice, despite lower yields. This is because of their similarity to human cells with respect to post-translational modifications and glycosylation patterns that cannot be adequately reproduced in the other systems (Browne & Al-Rubeai, 2007).

Despite the fact that significant improvements have been made to increase mAbs yields, mammalian cell culture still remains an expensive process with long development time (Browne & Al-Rubeai, 2007). Therefore, it is also crucial to optimize the mammalian cell *in vitro* production system in order to increase culture yields and decrease the duration and cost involved for the development and production of humanized mAbs.

Hence, in this research, using molecular and cell culture techniques, methodologies for the production of hum-C2 mAbs with high efficiency in terms continuity of production, lowered cost and increased speed were also optimized. In addition, high-throughput and automated systems were also utilized to facilitate the development and production phase of hum-C2 mAbs.

1.2 RESEARCH OBJECTIVES

The objectives of this research can be divided into 2 major parts: general and specific.

The general objective is:

- To develop procedures to produce humanized monoclonal antibodies against C2-antigen from monoclonal antibody of mouse origin with high efficiency in terms of continuity of production, lowered cost and increased speed.

The specific objectives are:

- To compare two methods of antibody humanization in terms of immunogenicity and functionality for the development of humanized anti-C2 monoclonal antibodies: a deimmunization method which uses a computer algorithm and IgBLAST software, and a logical approach method which uses only IgBLAST software.
- To acquire knowledge and expertise of using an automated and high-throughput selection system, ClonePix FL, to characterize the properties of the equipment for the selection of mammalian transfectoma cells secreting high level of humanized anti-C2 mAbs.
- To apply and optimize cytotechnology techniques for the serum-free adaptation of transfectoma cells, and also for the production, purification and characterization of humanized anti-C2 mAbs developed by both deimmunization and logical approach methods.
- To redevelop humanized anti-C2 monoclonal antibody using deimmunization method but using synthetic deoxyribonucleic acids (DNAs), pFUSE expression vectors and ubiquitous chromatin opening element (UCOE) expression vectors

instead. The productivity of both vectors in NS0 and Chinese hamster ovary (CHO) mammalian cells were compared.

1.3 THESIS OUTLINE

All experiments described in the chapters of this thesis have been published or recently submitted for publication in peer-reviewed scientific journals with the exception of chapter 3 (expression vectors construction). Therefore, each chapter is largely written in the format required by that journal and consists of its own body of scientific work which includes the associated introduction, material, method, result, discussion, and conclusion sections. Additional significant results which were not published are presented in appendices.

The research part of this thesis is divided into two sections:

I) Development, production and characterization of humanized anti-C2 mAbs (H1C2 and H2C2 mAbs) which consists of four chapters,

II) Optimization of the development and production phase of humanized anti-C2 monoclonal antibody (H1C2 mAb) which consists of one chapter.

Section I: Development, production and characterization of humanized anti-C2 mAbs (H1C2 and H2C2 mAbs)

- Chapter 3 describes the recombinant DNA technologies applied for the construction of pAH4602 and pAG4622 expression vectors harboring humanized anti-C2 mAbs variable regions using deimmunization method (H1C2

mAb) and logical approach method (H2C2 mAb). The deimmunization method was obtained through a technology transfer from Centre of Molecular Immunology, Cuba to Inno Biologics, Malaysia.

- Chapter 4 describes the transfection of the expression vectors harboring humanized anti-C2 mAb developed using deimmunization method (H1C2 mAb) from chapter 3 in NS0 mammalian cells. In this chapter, the optimization and characterization of the ClonePix FL system, a high-throughput automated colony picker, which was employed for the identification and isolation of transfectomas secreting high level of H1C2 mAbs is described.

- Chapter 5 discusses the application of cytotechnology techniques on the serum-free adaptation of the selected high-producing NS0 transfectomas acquired from chapter 4 and also the small-scale production and purification of H1C2 mAb using Äktaprime plus system.

- Chapter 6 describes how humanized mAbs were generated using the logical approach method (H2C2 mAb) as mentioned in chapters 3-5. Their functionality and potential immunogenicity in humans were then predicted by testing in non-human primates *Macaca fascicularis* and were compared to mouse anti-C2, chimeric anti-C2 and humanized anti-C2 mAb generated by deimmunization method (H1C2 mAb).

Section II: Optimization of the development and production phase of humanized anti-C2 monoclonal antibody (H1C2 mAb)

- From the experimental outcomes described in chapters 3-6, it was found that the H1C2 mAb which was developed using deimmunization method had lower immunogenicity compared to that of H2C2 mAb developed using logical approach method. Nevertheless, the use of overlapping-PCR mutagenesis to humanize mouse residues in both humanization methods frequently results in undesired mutations which cause the methods to be time-consuming and laborious. Besides that, the productivity of both H1C2 and H2C2 mAbs using monocistronic pAH4602 and pAG4622 expression vector and its expression using NS0 mammalian cells were relative low.

- Therefore, in chapter 7, we described the redevelopment of H1C2 mAb using the deimmunization method but with three major modifications. First, the overlapping-PCR mutagenesis was substituted with a method using synthetic DNA coding for the H1C2 mAb variable regions. Second the expression vectors used previously were substituted with pFUSE and UCOE expression vectors. Third, the H1C2 mAbs were expressed in NS0 and also in CHO mammalian cells. The transfectomas secreting high level of H1C2 mAbs were then isolated and characterized as described in chapters 4 and 5. Finally, the combination of expression vector and the cell line that results in the highest and most stable production of H1C2 mAbs were determined. This combination will be used for large-scale production and downstream applications of H1C2 mAbs.

1.4 THESIS FLOWCHART

Figure 1.1: Section I-Development, production and characterization of humanized anti-C2 monoclonal antibodies (Chapter 3-6)

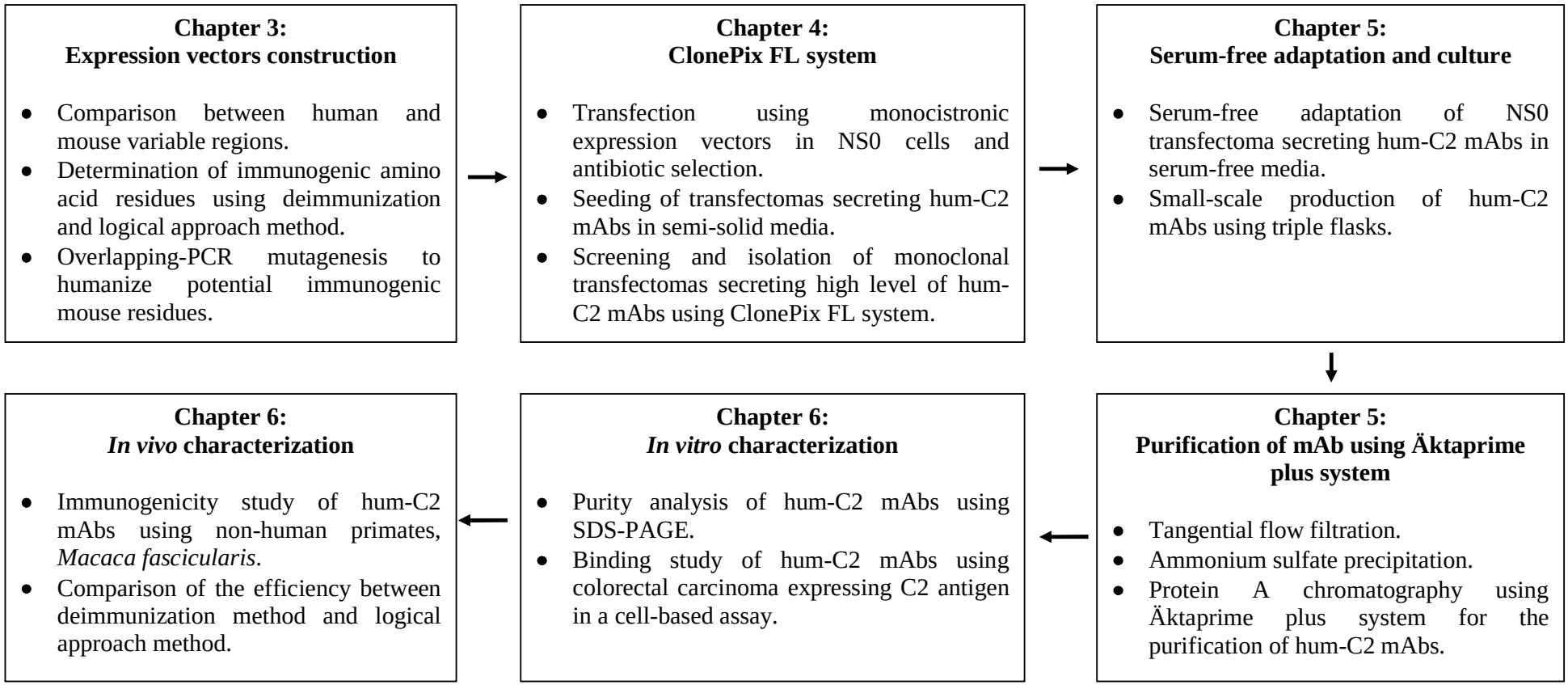

Figure 1.2: Section II- Optimization of the development and production phase of humanized anti-C2 monoclonal antibody (H1C2 mAb) (Chapter 7)