CHAPTER II

LITERATURE REVIEW

2.1 ANTIBODY

Antibodies are proteins produced by B-lymphocytes in the immune system in response to antigens, and function primarily to protect the body against invasion by foreign agents such as microbes.

Antibodies can be found as membrane-bound entities on B-cells or can be secreted into the circulation by plasma cells. The introduction of a specific antigen induces an interaction between the antigen and the membrane-bound antibody on a mature naive B-cell, as well as interactions with T-cells and macrophages and selectively induces the activation and differentiation of B-cell clones of corresponding specificity. In this process, the B-cell divides repeatedly and differentiates, generating a population of plasma cells and memory cells.

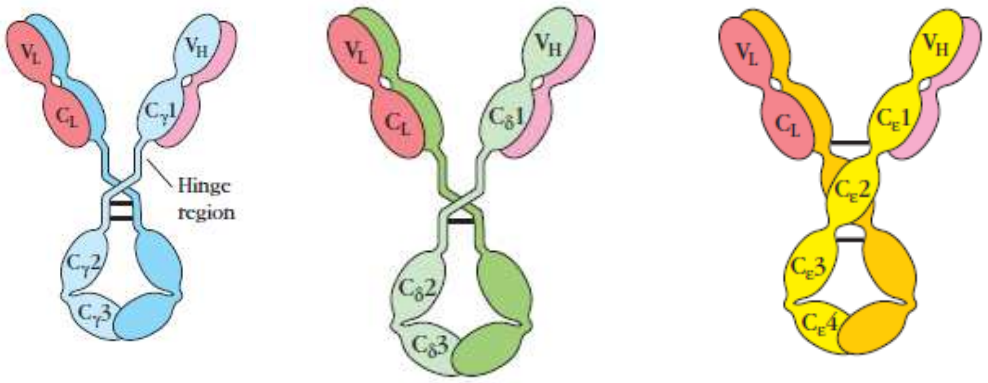
Plasma cells, which have lower levels of membrane-bound antibody than B-cells synthesize and secrete antibody. All clonal progeny from a given B-cell secrete antibodies with the same antigen-binding specificity (Goldsby et al. 2002). Secreted

antibodies from plasma cells circulate in the blood, where they serve as the effectors of humoral immunity by searching out and neutralizing antigens or marking them for elimination (Goldsby et al. 2002). The high specificity and affinity have led to the development of antibodies into diagnostic and therapeutic agents (Moore & Clayton, 2003).

In humans, there are five antibody classes, IgG, IgM, IgA, IgE, and IgD (Figure 2.1), which differ in the composition of the constant part of their heavy chains γ, μ, α, ε, δ, respectively (Goldsby et al. 2002). Minor differences in the amino acid sequences of α and γ heavy chains have led to further classification of the heavy chains into subisotypes that determine the subclass of antibody molecules they constitute.

There are also two subisotypes of α heavy chain in humans, α1 and α2 which constitute subclasses IgA1 and IgA2, respectively, and four subisotypes of γ heavy chains: γ1, γ2, γ3, and γ4 constituting subclasses IgG1, IgG2, IgG3, and IgG4, respectively (Figure 2.2). In this thesis, however, only the 150 kDa of subclass IgG1, which is the commonly used therapeutic antibody will be discussed and henceforth referred to as ‘antibody’.

a) IgG b) IgD c) IgE



d) IgA e) IgM

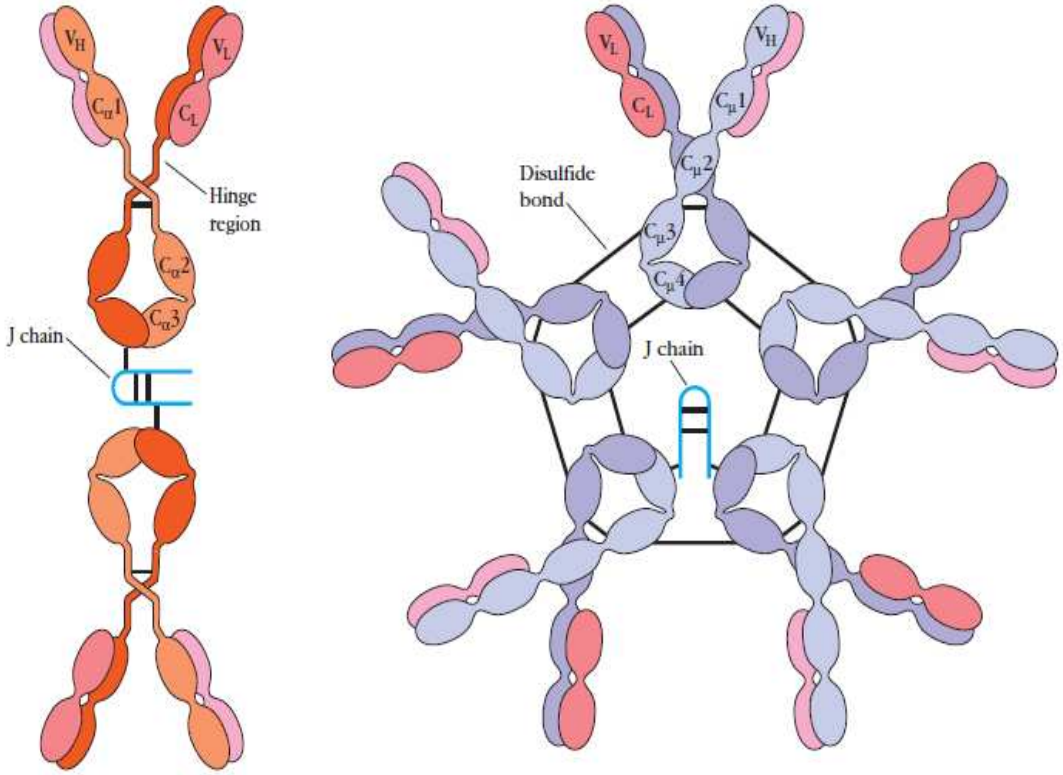


Figure 2.1: General structures of the five major classes of secreted antibody. Light chains are shown in shades of pink, disulphide bonds are indicated by thick black lines.

Source: Goldsby et al. 2002.

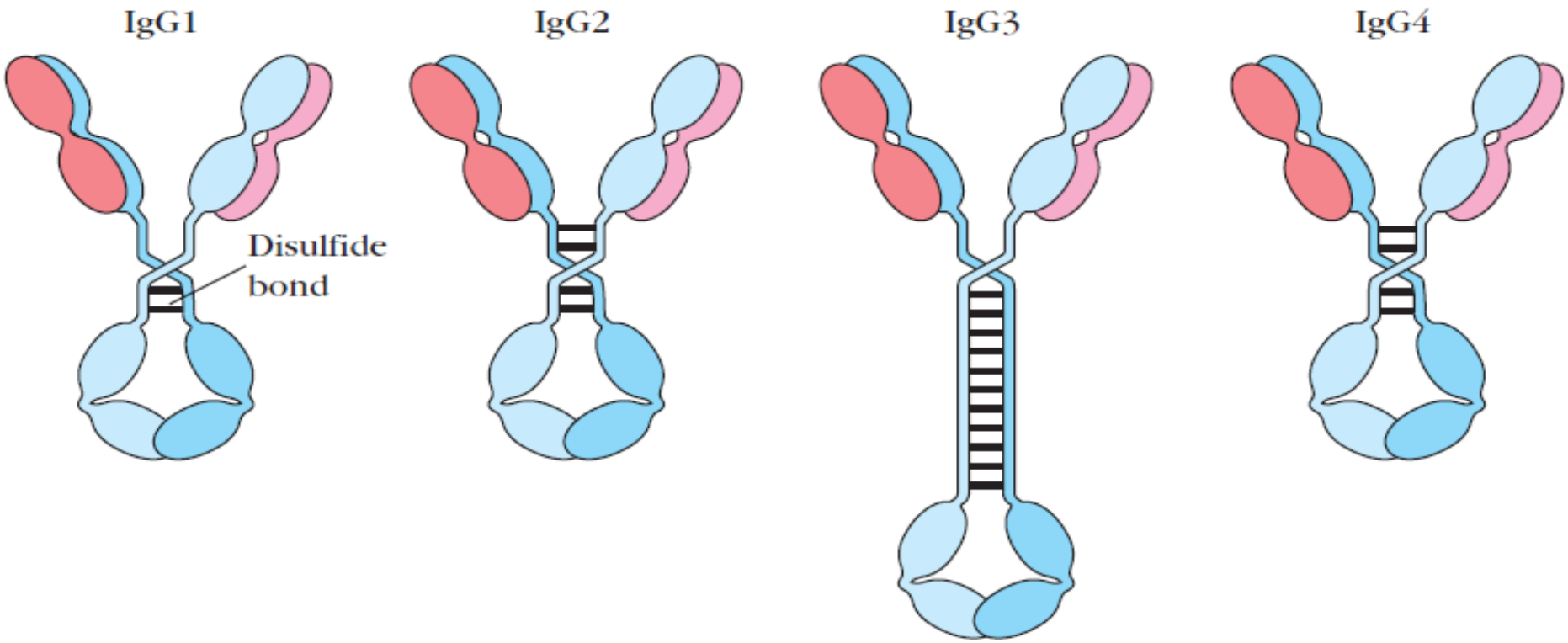


Figure 2.2: General structure of the four subclasses of human IgG. The subclasses differ in the number and arrangement of the inter-chain disulfide bonds (thick black lines) linking the heavy chains.

Source: Goldsby et al. 2002.

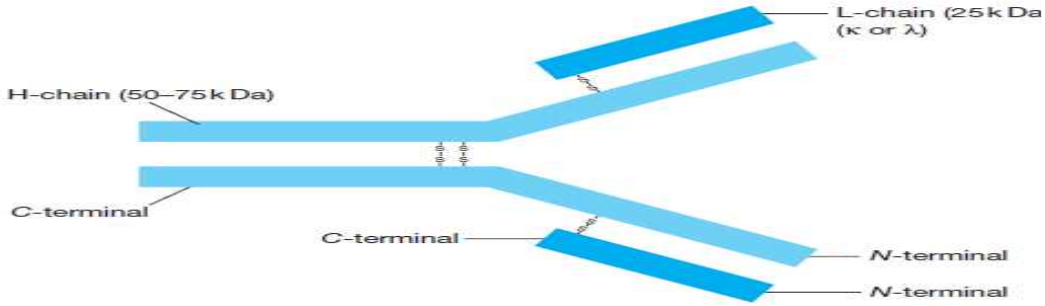
2.1.1 ANTIBODY STRUCTURE

The basic antibody molecule is depicted as a Y-shaped molecule consisting of four protein subunits (Figure 2.3a). The two longer subunits are called heavy (H)-chains and are identical to each other. They have a molecular mass of 50 kDa. The two shorter subunits are also identical to each other; they are called light (L)-chains and have a molecular mass of about 25 kDa. The light chain consists of variants κ and λ. They are very similar in structure but are coded by different genes and are without any known functional differences (Bengten et al. 2000). An individual antibody molecule usually contains two H-chains and two κ-chains or two λ-chains. The heavy chains are linked to each other and to the light chains by disulphide bridges.

Further analysis of antibody structure shows that both the heavy and the light chains have repeating substructures called domains (Figure 2.3b). These domains are regions of approximately 110 amino acids within the heavy and light chains and are flanked by intra-chain disulphide bridges.

The amino-terminal regions of heavy and light chains, which vary greatly among antibodies with different specificities, are called variable (V)-regions; VH for heavy chain and VL for light chain. Within the V-regions, sequence variability is concentrated in several hypervariable regions. These hypervariable regions, which constitute the antigen-binding site of an antibody, are called complementarity-determining regions (CDRs). The remaining domains of VH and VL, which exhibit far less variation, are called the framework regions (FWR).

a)



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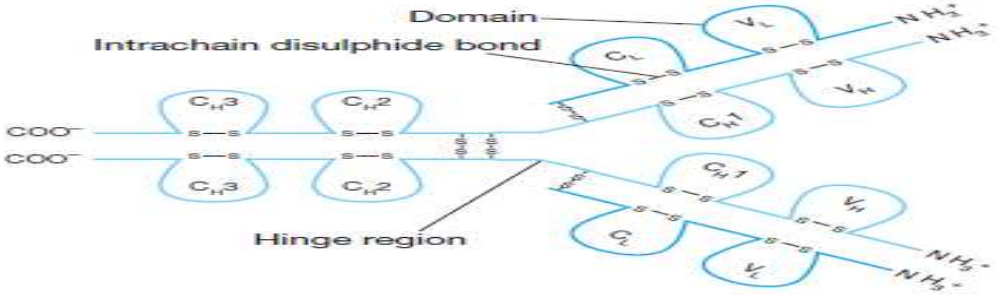

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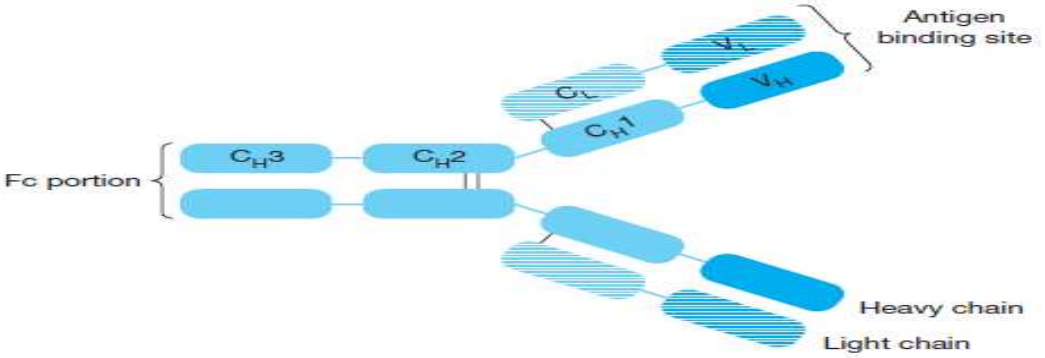

Figure 2.3: Antibody structure. (a) Each antibody molecule contains two identical larger (heavy) and two identical smaller (light) chains. (b) The heavy and light chains contain domains that flanked by intrachain disulphide bonds. (c) The antibody has two identical Fabs and one Fc domain.

Source: Wood, 2006.

The regions of relatively constant sequences beyond the variable regions are called constant (C) regions, CH for heavy chain and CL for light chain. The heavy chain of the antibody has six constant domains and the light chain has two. The heavy chain constant domains are called CH1, CH2, and CH3; the light chain domain is called CL. Between the CH1 and CH2 domains of the heavy chain is a region containing several prolines which makes this part of the antibody quite flexible and it is therefore known as the hinge region (Wood, 2006).

The antibody can also be divided into three functional domains (Figure 2.3c); two antigen-binding domains with identical antigen specificity, connected by a highly flexible hinge region to a domain that are unable to bind to antigen but are found to crystallize during cold storage. Due to these properties, the antigen-binding domains are known as Fab (Fragment-antigen binding) and the non-antigen binding domain as Fc (fragment-crystallizable).

Both Fab and Fc domains play major biological roles: 1) the Fab domain is involved in the binding function for the recognition of foreign antigens such as toxins, viruses, exposed molecules on the surface of pathogenic organisms and differentiated cells; 2) the Fc domain is involved in effector functions which results in the elimination or inactivation of the foreign antigen or the cell marked by the presence of that antigen (Roitt, 1993). The ability to specifically discriminate different antigens has made antibodies an indispensable tool in diagnostic medicine and as therapeutic agents of high potential.

2.1.2 THERAPEUTIC ANTIBODIES

Therapeutic antibodies such as anticancer antibodies constitute a growing family of novel molecules applied in the treatment for solid and hematological cancers. The long half-life, low toxicity and high affinity and specificity of mAbs are only a few of the advantages that make them attractive potential therapeutic agents (Zafir-Lavie et al. 2007). Antibodies may possess multiple anticancer therapeutic activities in directing cytotoxic effects to a tumor cell by direct and indirect mechanisms.

Direct therapeutic effects of monoclonal antibodies involve the induction of apoptosis, inhibition of proliferation, blockage of growth factors or growth factor receptors and interference of angiogenesis which usually lead to tumor cell death. Therapeutic antibodies may alter signal transduction within the tumor cell by targeting growth factor receptors, which are over-expressed in a number of malignancies. As their activation under normal condition promotes cellular survival and proliferation, therefore it follows that their over-expression promotes tumor cell growth.

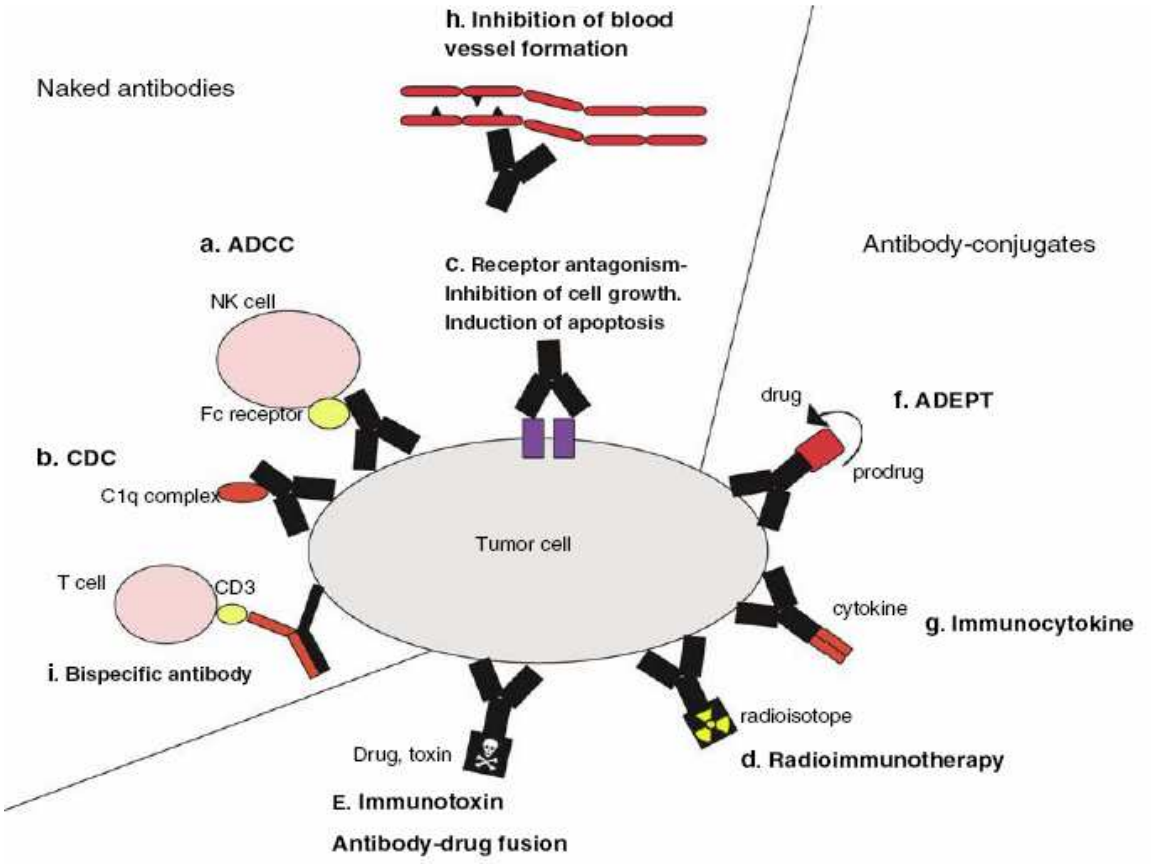

Figure 2.4: Anti-tumor mechanisms of action. Most mAbs interact with components of the immune system through ADCC (a) or CDC (b). Antibodies can also alter signal transduction within the tumor cell, and this result in the induction of apoptosis and/or inhibition of growth by acting as antagonists (c). When fused to radioisotopes (d), drugs or toxins (e), mAbs can directly kill tumor cells or activate prodrugs specifically within the tumor (f). Fusion of antibodies to cytokines (g) can directly stimulate anti-tumor immune response. Antibodies specific to unique antigens presented on new blood vessels can prevent angiogenesis (h). Bispecific T-cell engager molecules can redirect cytotoxic T-cells against target cells (i).

Source: Zafir-Lavie et al. 2007.

By diminishing signaling through these receptors, mAbs have the potential to normalize growth rates, activate the apoptosis pathway and desensitize tumor cells to cytotoxic agents. Examples are antibodies that target members of the epidermal growth factors receptors (EFGR) family, which work by physically blocking the interaction between the receptor and its activating ligand and by sterically preventing the receptor from acquiring the extended conformation required for dimerization (Ferguson, 2004).

Another example of potent inhibitors of signal transduction is anti-HER2 antibody which allows ligand binding to occur but sterically hinder HER2 recruitment in forming heterodimers with other HER receptors. This results in the inhibition of signaling by HER2-based heterodimers (Albanell et al. 2003). It has been proposed that a number of antibodies that are currently in use for cancer treatment may directly induce apoptosis in tumor cells (Shan et al. 2000).

In terms of indirect therapeutic effects, monoclonal antibodies may act by interaction with the components of the immune system through the antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC). ADCC is triggered when the Fab domains bind to specific antigen on the surface of target cells, and the Fc domain interacts with Fc receptor on the effector cells, such as natural killer cells and macrophages. This interaction activates the effector cells and lysis of the target cell is accomplished.

Besides ADCC, antibodies also utilize CDC as their anti-tumor activity. It has been proposed that antibodies are able to direct CDC via the classical complement-activation pathway. This pathway is triggered when the C1 complex which is comprised of three different proteins, binds the antibody-antigen complex. Following a cascade of

complement activation, a membrane attack complex is formed, and lysis, opsonization and immune complex clearance is achieved.

Antibodies have also been used extensively in clinical trials to direct cytotoxic agents towards the target tumor cells and these agents include radioisotopes, toxins, enzymes/prodrugs and cytokines. The rationale of the immunoconjugate approach is that it takes advantage of the specificity exhibited by antibodies to deliver cytotoxic agents directly, and higher local concentrations of drug or radiation at the tumor site would then increase tumor cell damage with less toxicity to normal cells.

Fusion of antibodies to major histocompatibility complex (MHC) molecules or cytokines can directly stimulate anti-tumor immune response. Finally, mAbs can be used synergistically with chemotherapeutic agents. Hence, the use of immunoconjugates to deliver drugs specifically to a tumor has a potential in improving anti-tumor efficacy and reducing systemic toxicity. The mechanisms of elimination of tumor cells by unconjugated (naked) antibody and conjugated antibody are demonstrated in Figure 2.4.

2.1.3 MONOCLONAL ANTIBODY

Antibodies can be produced either as monoclonal or polyclonal antibodies as illustrated in Figure 2.5. Most natural antigens have multiple epitopes, hence the exposure of the mouse to the antigen results in the generation of many B-cells producing antibodies against different epitopes of the antigen.

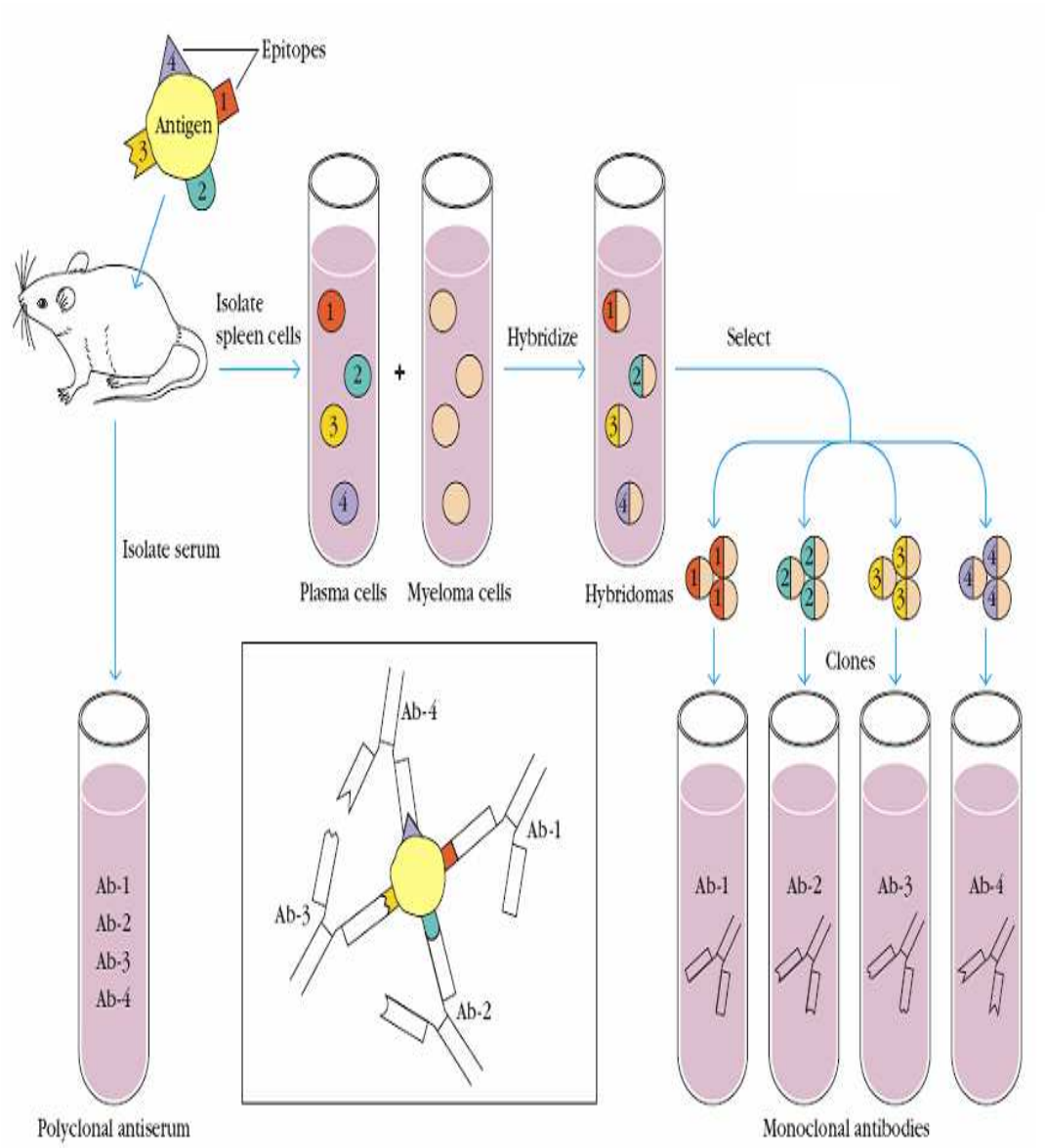

Figure 2.5: Production of polyclonal and monoclonal antibodies. The conventional polyclonal antiserum produced in response to a complex antigen contains a mixture of monoclonal antibodies, each specific for one of the four epitopes shown on the antigen. In contrast, a monoclonal antibody, which is derived from a single plasma cell, is specific for one epitope on a complex antigen.

Source: Goldsby et al. 2002.

A mixture of antibodies that recognize different epitopes on the same antigen are categorized as polyclonal. For many types of studies involving antibodies, monoclonal antibodies are preferable to polyclonal antibodies. However, the biochemical purification of monoclonal antibodies from the serum is not feasible, because the concentration of any given antibody is quite low and there is lot-to-lot variation.

For this reason, a methodology was developed to immortalize the plasma cells in order to obtain usable quantities of monoclonal antibody. Each plasma cell produces only one type of antibody, i.e. a monoclonal antibody. However, large-scale production of specific monoclonal antibodies from cell cultures of B-lymphocytes is not feasible as normal B-lymphocytes do not grow indefinitely in culture.

This limitation can be avoided by fusing normal B-lymphocytes with immortal transformed lymphocytes called myeloma to produce immortal mAb-producing hybridoma cells that can be maintained in culture *in vitro* for extended period of time. This 'hybridoma technology' developed by Kohler and Milstein (1975) allows the isolation and production of mouse monoclonal antibodies having specificity for a single antigen epitope such as a protein, carbohydrate and nucleic acids.

However, when most mouse mAbs were administered in human, many risks and limitations were discovered. First, the use of mouse mAbs elicited an AAR known as HAMA in treated patients (Table 2.1). In patients with AAR, increased incidences of immune complex syndrome, allergic reactions and infusion reactions had been reported (Baert et al. 2003). Second, the effector functions of mouse mAbs also proved to be less efficient in the human context. Third, their biological half-lives were shorter compared to human mAbs. These factors greatly limited their usefulness.

Although some human cell lines capable of producing human hybridomas have been described, for instance SK-007 (Olsson & Kaplan, 1980), GM1500 (Croce et al. 1980), LICR-LON-Hmy2 (Edwards et al. 1982) and Karpas 707 (Karpas et al. 1982), the overall experience remains disappointing. The problems that arose are largely due to the use of mouse myeloma as a fusion partner for human β-lymphocytes which often leads to the preferential loss of human chromosomes and instability of the hybrids. In addition, for ethical reasons it is not possible to immunize a human volunteer with an experimental antigen and *in vitro* immunization was also not able to solve the problem due to predominant IgM responses.

As an alternative procedure, antigen-specific B-lymphocytes were isolated from the peripheral blood of human donors and immortalized by Epstein-Barr virus (EBV) to establish permanent cell lines (Steinitz et al. 1977). Unfortunately, the production rates of the cell lines were low and decreased with time. Furthermore, the EBV transformed cell lines were extremely difficult to clone. The EBV hybridoma technique (Kozbor & Roder, 1981) in addition, is also complex and often leads to unstable hybridomas that require repeated re-cloning.

Table 2.1: Reported AAR; human anti-mouse antibody (HAMA) responses

Antibody (synonyms)	Indication	% of patients with AAR	References
2h4 and 5d3	Nasopharyngeal carcinoma	100	Li et al. 2002
Anti-Lym mAb	B-cell malignancies	38	O'Donnell et al. 2000
10-3D2	Breast tumor	14	Hnatowich et al. 1992
131I-labeled anti-HCC mAb/Hepama-1	Hepatocellular carcinoma	34	Zeng et al. 1994
131I-T101	Cutaneous T-cell lymphoma	100	Goldman-Leikin et al. 1988
14G2a	Refractory melanoma, neuroblastoma or osteosarcoma	89	Murray et al. 1994
16H5	Rheumatoid arthritis	50	Horneff et al. 1991
17-1A	Colorectal carcinoma	100	Frodin et al. 1992
A7-NCS	Colorectal carcinoma, pancreatic carcinoma	100	Takahashi et al. 1992
Anti-CEA antibody fragments-conjugated to bacterial enzyme	CEA-bearing tumors	100	Sharma et al. 1992

Table 2.1: continued

Anti-CEA, anti-In-DTPA bispecific Fab'-Fab	Colorectal carcinoma (imaging)	64	Le Doussal et al. 1993
Anti-melanoma or anti-CEA mAbs	Melanoma, colorectal carcinoma (imaging)	83	Endo et al. 1990
Arcitumomab	Colorectal carcinoma for imaging	0	Wegener et al. 2000
B43-Genistein	B-lineage acute lymphoblastic leukemia	33	Uckun et al. 1999
B72.3	Colorectal carcinoma	44	Winzelberg et al. 1992
B-C7	Septic shock	100	Boillot et al. 1995
B-E8	Myeloma, renal cell carcinoma	75	Legouffe et al. 1994
BrE-3, 111In-MX-DTPA	Human ductal breast cancer	83	DeNardo et al. 1997
BW 250/18299Tc-labeled	Detection of inflammatory lesions (imaging)	1	Berberich et al. 1992
BW 431/2699Tc-labeled	Colorectal and lung adenocarcinoma (imaging)	33	Hertel et al. 1990
BW 494/BI 51.011	Pancreatic cancer	94	Schulz et al. 1988
CCR086 indium-111-labeled	Detection of colorectal carcinoma metastases (imaging)	80	Abdel-Nabi et al. 1990
CYT-103 111In-labeled anti-TAG-72 mAb	Colorectal carcinoma (imaging)	42	Muxi et al. 1993
D612	Metastatic gastrointestinal cancer	86	Saleh et al. 1993

Table 2.1: continued

E5/Edobacomab	Gram negative sepsis	20	Hwang & Foote, 2005
HMFGI, HMFG2, HI7E2, B72.3	Ovarian cancer radioimmunotherapy	100	Riva et al. 1989
HRS-3/A9	Hodgkin's disease	46	Renner et al. 2000
IgM mAbs,WM63(CD48) and WM66	Chronic lymphocytic leukemia	0	Greenaway et al. 1994
Ior EGF/r3	Gliomas or meningiomas	89	Crombet et al. 2001
LL2	Non-Hodgkin's lymphoma	19	Vose et al. 2000
L6	Adenocarcinoma	64	Ziegler et al. 1992
M195	Myeloid leukemias, myelodysplastic syndromes	37	Schwartz et al. 1993
MAb with high human immunodeficiency virus type 1 neutralizing titers	Human immunodeficiency virus	73	Hinkula et al. 1994
OC-TR bispecific mAb	Intraperitoneal treatment of ovarian cancer	100	Lamers et al. 1995
OC125	Ovarian cancers	100	Maher et al. 1992
OKB7I131-labeled	CD21-positive, non-Hodgkin's lymphoma	75	Czuczman et al. 1993
OKT3	Graft rejection	86	Hammond et al. 1993

Table 2.1: continued

OV-TL 3	Ovarian cancer	40	Hwang & Foote, 2005
I131-tositumomab/Bexxar	Non-Hodgkin's lymphoma	8	Kaminski et al. 2001
YTH 24.5 and YTH 54.12	Pre-treatment of donor organs for transplantation	5	Watts et al. 1995
ZCE 025	Colorectal carcinoma	64	Abdel-Nabi et al. 1992
ZME 018 and 96.5	Melanoma or basal cell carcinoma	88	Frontiera et al. 1989

2.2 ANTIBODY ENGINEERING

Three main forms of engineered monoclonal antibodies as illustrated in Figure 2.6 have been devised in an attempt to reduce immunogenicity associated with mouse mAbs and to improve their pharmacokinetic and effectors functions in patients.

2.2.1 CHIMERIC MONOCLONAL ANTIBODY

Chimeric mAbs are made by joining the variable domains of a mouse mAb to the constant domains of a human antibody using DNA recombinant techniques, thus reducing the immunogenicity and ensuring the efficient recruitment of human effectors function (Boulianne et al. 1984). Chimeric mAbs also have longer plasma half-lives compared to mouse mAbs. However, the chimeric antibodies do still elicit an AAR referred to as human anti-chimeric antibody (HACA) response due to the presence of mouse variable domains (Table 2.2).

2.2.2 HUMANIZED MONOCLONAL ANTIBODY

The immunogenicity of chimeric mAbs can be further reduced by CDR-grafting method, whereby the six CDR regions of the mouse light and heavy chain are grafted on to the human immunoglobulin framework region (Jones et al. 1986). Thus, the resulting humanized antibodies contain approximately 90% of human amino acid sequences.

Figure 2.6: Engineered forms of monoclonal antibodies. The percentage of original mouse amino acid residues were reduced to lower the immunogenicity associated with mouse monoclonal antibodies.

Source: Wood, 2006.

Table 2.2: Reported AAR; human anti-chimeric antibody (HACA) responses

Antibody (synonyms)	Antigen; Indication	% of patients with AAR	References
Rituximab/Rituxan	CD20; B-cell non-Hodgkin's lymphoma	0	Piro et al. 1999
Abciximab	β3 intergrin of the GPIIb/IIIa and $\alpha_v\beta_3$ receptors on human platelets; adjunct to percutaneous transluminal coronary angioplasty or atherectomy (PCTA) for the prevention of acute cardiac ischemic complications	19.0	Tcheng et al. 2001
Basiliximab/Simulect	CD25; prophylaxis/treatment of organ/hematopoietic stem cell transplant or graft versus host disease	1.4	Hwang & Foote, 2005
Infliximab/Remicade	TNFα; Crohn's disease, rheumatoid arthritis	61	Baert et al. 2003
111In-labeled c-Nd2	Pancreatic cancer (imaging)	0	Sawada et al. 1999
131I-labeled chimeric L6	Breast cancer	67	Richman et al. 1995
cG250	Clear cell renal cancer	0	Varga et al. 2003; Steffens et al. 1997
ch 14.18	Ganglioside GD 2; neuroblastoma	0	Handgretinger et al. 1995

Table 2.2: continued

chA7Fab	CEA expressing tumors	71	Yata et al. 2003
Chimeric OC/TR	Bispecific mAb fragment against CD3 and human folate-binding protein (FBP) on non-mucinous ovarian carcinomas; ovarian carcinoma	50	Luiten et al. 1997
cMOv18	Ovarian carcinoma	0	Buist et al. 1995; Van Zanten-Przybysz et al. 2002
cM-T412	CD4: rheumatoid arthritis	60	Choy et al. 1998
Anti-CEA mAb/ct84.66	Metastatic CEA-producing malignancies	7	Wong et al. 1995
(186)Re-labeled chimeric U36	CD44v6; head and neck cancer	40	Colnot et al. 2000
Cetuximab/Erbitux	Epidermal growth factor receptor; colorectal cancer	5	Hwang & Foote, 2005

This method has the advantage of conserving the structure of the antibody variable regions, while using the human framework residues as a scaffold to support the mouse CDR loops which will determine antigen specificity. Although this approach can be successful for some antibodies, it often results in the loss of affinity and a number of framework residues have to be back mutated to the corresponding mouse ones to restore binding (Riechmann et al. 1988; Padlan, 1991). In addition, a rare AAR referred to as human anti-human antibody (HAHA) response was observed with CDR-grafted humanized mAbs (Table 2.3) (Nechansky, 2010).

The incidence of AAR; HAMA (Table 2.1), HACA (Table 2.2) and HAHA (Table 2.3) can be grouped into three operational categories (Figure 2.8); marked, tolerable, and negligible. The immunogenicity of mAbs was classified as “marked” by Hwang and Foote (2005), if present in more than 15% of patients, hence these mAbs were usually clinical failures and regulatory concerns were likely to preclude clinical use except a one-time use as radio-immunoconjugates. Approximately 85% of mouse mAbs, were categorized as marked, followed by 40% of chimeric mAbs and 9.1% of humanized mAbs. On the contrary, 54.5% of humanized and 33.3% chimeric mAbs, respectively, were shown as having negligible immunogenicity compared to only 7.15% of mouse mAbs. Similarly, 36.4%, 26.7% and only 7.15% of the humanized, chimeric and mouse mAbs, respectively, showed tolerable immunogenicity. Immunogenicity of mAbs was classified as tolerable if detectable in 2-15% of patients and is essentially flawed, though use was arguably warranted for catastrophic or life-limiting disease. Since the mAbs were classified as ‘negligible’ immunogenicity if the AAR was reported in less than 2% of patients, therefore immunogenicity this low represents an ideal, with minimum need for concern about safety.

Table 2.3: Reported AAR; human anti-human antibody (HAHA) responses

Antibody (synonyms)	Antigen; Indication	% of patients with AAR	References
Alemtuzumab/Campath-1H	CD52; B-cell chronic lymphocytic leukemia (B-CLL)	1.9	Hwang & Foote, 2005
Daclizumab/Zenapax	CD25; prophylaxis of acute organ rejection in patients receiving renal transplants, to be used as part of an immunosuppressive regimen that includes cyclosporine and corticosteroids	34	Hwang & Foote, 2005
Palivizumab/Synagis	Respiratory syncytial virus; prophylaxis in pediatric patients at high risk of RSV disease	1	Hwang & Foote, 2005
Transtuzumab/Herceptin	HER2/neu (human epidermal growth factor 2); metastatic breast cancer with tumors overexpressing the HER2 protein	0.1	Hwang & Foote, 2005
16.88/CTA 16.88	CTA 16.88-bearing epithelial-derived tumors including carcinomas of the colon, pancreas, breast, ovary, and lung	0	De Jager et al. 1993
88BV59/CTA 16.88	CTA 16.88-bearing epithelial-derived tumors (different epitope)	3.7	De Jager et al. 1993; Serafini et al. 1998
hu-A33	“A33” colonic epithelium Ag; colon cancer	49	Hwang & Foote, 2005

Table 2.3: continued

Bivatuzumab/BIWA 4	CD44v6; head and neck squamous cell carcinoma	10	Colnot et al. 2003; Borjesson et al. 2003
Humicade/CDP571	TNFα; Crohn's disease	7	Hwang & Foote, 2005
Gemtuzumab Ozogamicin/Mylotarg	CD33; acute myeloid leukemia	2.9	Jurcic et al. 2000
Hu23F2G	CD11/CD18; acute non-infectious inflammatory disorders mediated predominantly by neutrophils, e.g., multiple sclerosis	0	Bowen et al. 1998
Hu2PLAP	Placental alkaline phosphatase	0	Hird et al. 1991
Hu5c8	CD154 (CD40 ligand); hemophilia (by suppressing anti-factor VIII antibodies)	0	Ewenstein et al. 2000
HuBrE-3	Breast cancer	14	Kramer et al. 1998
HuM291	CD3; renal transplant and graft versus host disease	0	Carpenter et al. 2002
Adalimumab/Humira	TNFα; developed by phage display of a human antibody library	12	Hwang & Foote, 2005
Natalizumab	α4 integrin; Crohn's disease	7	Ghosh et al. 2003
Omalizumab	Allergic asthma	0	Nayak et al. 2003
Efalizumab/Raptiva	CD11a; psoriasis	6.3	Hwang & Foote, 2005

Table 2.3: continued

Bevacizumab/Avastin, rhu anti-VEGF mAb	VEGF expressing solid tumors	0	Margolin et al. 2001; Gordon et al. 2001
Cantuzumab Mertansine	CanAg glycoform of MUC1; colorectal cancer	0	Tolcher et al. 2003
Vitaxin	integrin $\alpha_4\beta_3$ (vitronectin receptor); metastatic cancer	0	Posey et al. 2001; Gutheil et al. 2000

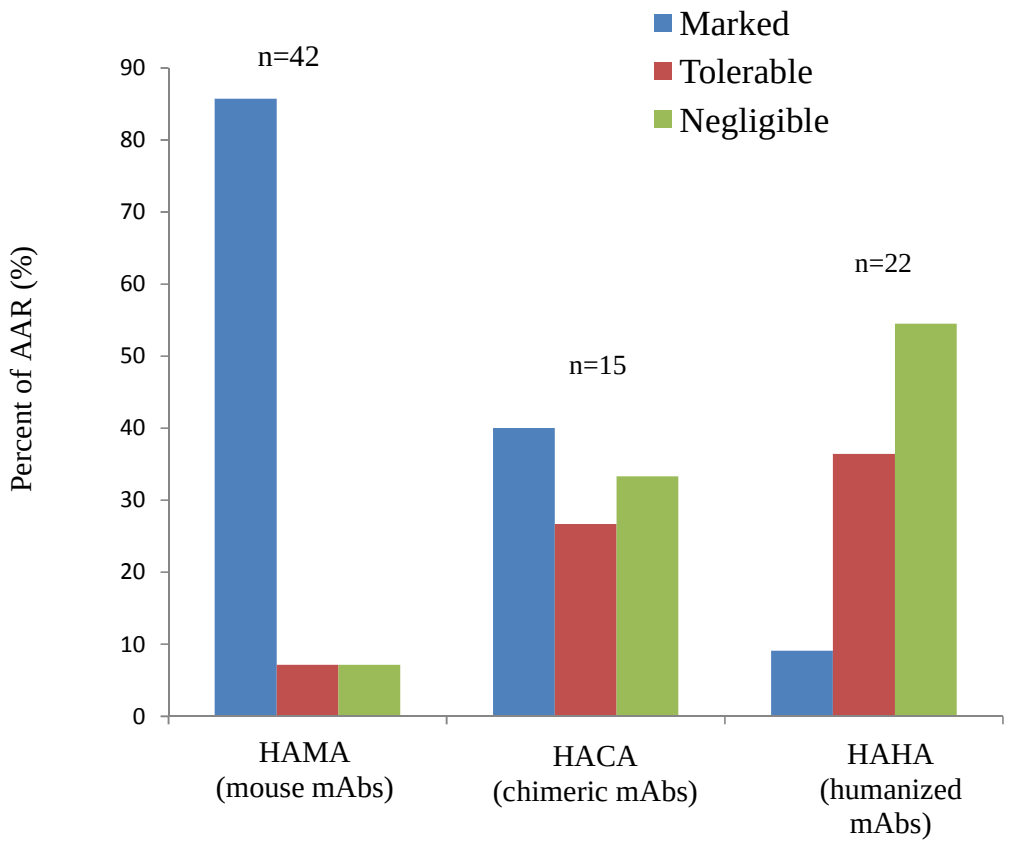

Figure 2.7: Immunogenicity of mouse, chimeric and humanized monoclonal antibodies administered to humans. The degree of AAR was classified as marked, tolerable and negligible if detectable in more than 15%, between 2-15%, and less than 2% of patients, respectively.

Source: Data adapted from Hwang & Foote (2005).

2.2.3 FULL HUMAN ANTIBODY

The chimeric and humanized mAbs are both able to overcome short biological half-lives and the lack of effector functions of parental mouse mAbs. These two engineered mAbs have prolonged biological half-lives and ensuring the efficient recruitment of human effector functions (Zhang et al. 2007). In terms of immunogenicity, development of chimeric mAbs reduces the immunogenicity of mouse mAbs while CDR-grafting of humanized mAbs reduces it further (Hwang & Foote, 2005).

Therefore, fully human mAbs were anticipated to be non-immunogenic and thus would allow repeated administration in human without any concern of AAR (Yang et al. 2001). Nevertheless, even fully human mAbs have also been shown to induce AAR (Clark, 2000). Humira (adalimumab) a fully human mAb developed by phage display technology, was approved by FDA, USA, for the treatment of rheumatoid arthritis in 2002; however, it does incur immunogenicity in 12% of the treated human population when used alone (Hwang & Foote, 2005).

Hence, although the generation of fully human mAbs is possible through the expression of human immunoglobulin genes in transgenic animals or by phage display technologies, a reduced immunogenicity of fully human antibodies cannot be assumed (Clark, 2000).

2.3 APPROVED THERAPEUTIC MONOCLONAL ANTIBODIES

To date, a total of 30 mAbs have been approved for therapeutic use in humans. The fact that only four are mouse mAbs while the rest are engineered forms of mAbs clearly shows the success of engineered mAbs in overcoming the disadvantages and limitations of parental mouse mAbs. From the 26 engineered forms of mAbs; five are chimeric mAbs; twelve are humanized mAbs and nine are fully human mAbs.

The nomenclature of mAbs has been devised for assigning generic or non-proprietary names. In the United States Adopted Names (USAN) system, all mAbs names end with the suffix -mab, whereas different infixes are used to differentiate the agents depending on the structure of the therapeutic mAbs. The infix immediately preceding the -mab suffix e.g o (muromomab), xi (abciximab), zu (daclizumab) and u (adilimumab) denote mouse, chimeric, humanized and human antibodies respectively. The properties of all the 30 approved mAbs are summarized in Table 2.4.

Table 2.4: Approved monoclonal antibodies for therapeutic use

Trade name	USAN	Indication	Year approved	Use	Type
Orthoclone OKT3	Muromomab	Immunologic	1986	Therapeutic	Mouse
ReoPro	Abciximab	Cardiac	1994	Therapeutic	Chimeric
Rituxan	Rituximab	Oncologic	1997	Therapeutic	Chimeric
Zenapax	Daclizumab	Immunologic	1997	Therapeutic	Humanized
Simulect	Basiliximab	Immunologic	1998	Therapeutic	Chimeric
Synagis	Pavilizumab	Infectious disease	1998	Therapeutic	Humanized
Remicade	Infliximab	Immunologic	1998	Therapeutic	Chimeric
Herceptin	Trastuzumab	Oncologic	1998	Therapeutic	Humanized
Mylotarg	Gemtuzumab ozogomicin	Oncologic	2000	Therapeutic	Humanized
Zevalin	Ibritumomab tiuxetan	Oncologic	2002	Therapeutic	Mouse
Humira	Adilimumab	Immunologic	2002	Therapeutic	Human

Table 2.4: continued

Xolair	Omalizumab	Immunologic	2003	Therapeutic	Humanized
Bexxar	Tositumomab	Oncologic	2003	Therapeutic	Mouse
Raptiva	Efalizumab	Immunologic	2003	Therapeutic	Humanized
Erbitux	Cetuximab	Oncologic	2004	Therapeutic	Chimeric
Avastin	Bevacizumab	Oncologic	2004	Therapeutic	Humanized
Tysabri	Natalizumab	Immunologic	2004	Therapeutic	Humanized
Lucentis	Ranibizumab	Ophthalmologic	2006	Therapeutic	Humanized
Vectibix	Panitumumab	Oncologic	2006	Therapeutic	Human
Soliris	Eculizumab	Immunologic	2007	Therapeutic	Humanized
Cimzia	Certolizumab pegol	Immunologic	2008	Therapeutic	Humanized
Simponi	Golimumab	Immunologic	2009	Therapeutic	Human
Ilaris	Canakinumab	Immunologic	2009	Therapeutic	Human
Removab	Catumaxomab	Oncologic	2009	Therapeutic	Mouse
Stelara	Ustekinumab	Immunologic	2009	Therapeutic	Human

Table 2.4: continued

Arzerra	Ofatumumab	Oncologic	2009	Therapeutic	Human
RoActemra	Tocilizumab	Immunologic	2010	Therapeutic	Humanized
Prolia	Denosumab	Immunologic	2010	Therapeutic	Human
Benlysta	Belimumab	Immunologic	2011	Therapeutic	Human
Yervoy	Ipilimumab	Oncologic	2011	Therapeutic	Human[1]

[1] USAN: United States adopted names

2.4 DNA RECOMBINANT TECHNOLOGY IN ANTIBODY ENGINEERING

The techniques of molecular biology, specifically DNA recombinant technologies are extensively used to design, engineer and express engineered mAbs from mouse mAbs developed using hybridoma technology. The growing knowledge of antibody gene structure and regulation has also enabled the construction of engineered mAbs. It is now possible to design and construct genes that encode mAbs in which the variable regions are acquired from one species and the constant regions from another species.

The mechanics of producing engineered mAbs using the DNA recombinant techniques are relatively straightforward and it begins with the isolation of ribonucleic acid (RNA) coding the variable regions from hybridoma cells secreting mouse mAbs. Then, the complementary DNAs (cDNA) of the variable regions are obtained by Reverse Transcription Polymerase Chain Reaction (RT-PCR).

The variable regions of the antibody genes are then cloned into an appropriate expression vector followed by the transfection into mammalian cell line for the expression of the engineered mAbs. The expression vectors used are usually monocistronic or bicistronic vectors containing the promoter, leader and the constant-region exons from a human antibody gene (Figure 2.8).

Monocistronic vectors are used to express either the heavy or light chain of the mAb; hence for the expression of a complete engineered mAb, two monocistronic vectors expressing the heavy and light chain are required. On the other hand, a single bicistronic

vector is sufficient for the expression of a complete mAb, as it can express both the heavy and light chain of a mAb simultaneously.

The combination of mouse variable regions with human constant regions results in the expression of chimeric mAb (Figure 2.8). Therefore, its antigenic specificity which is determined by the variable region remains the same since it is derived from the mouse DNA. Meanwhile its isotype which is determined by the constant region, is that of human since it is derived from the human DNA. Because the constant regions of these chimeric antibodies are encoded by human genes, the resulting chimeric antibodies have fewer mouse antigenic determinants and therefore are less immunogenic in humans compared to that of mouse mAbs.

To further reduce the immunogenicity of chimeric mAbs, humanized mAbs are developed by substituting the mouse variable regions with humanized variable regions. For the expression of humanized mAbs, the humanized variable regions are similarly cloned into monocistronic or bicistronic expression vectors and transfected in mammalian cells. The resulting humanized mAbs contain minimum mouse amino acids in its variable regions which are sufficient to retain its original specificity and functionality. Since the reduction of mouse regions in humanized mAbs usually results in the reduction of its immunogenicity in humans, therefore humanized mAbs are preferred as human therapeutics compared to its corresponding mouse and chimeric mAbs.

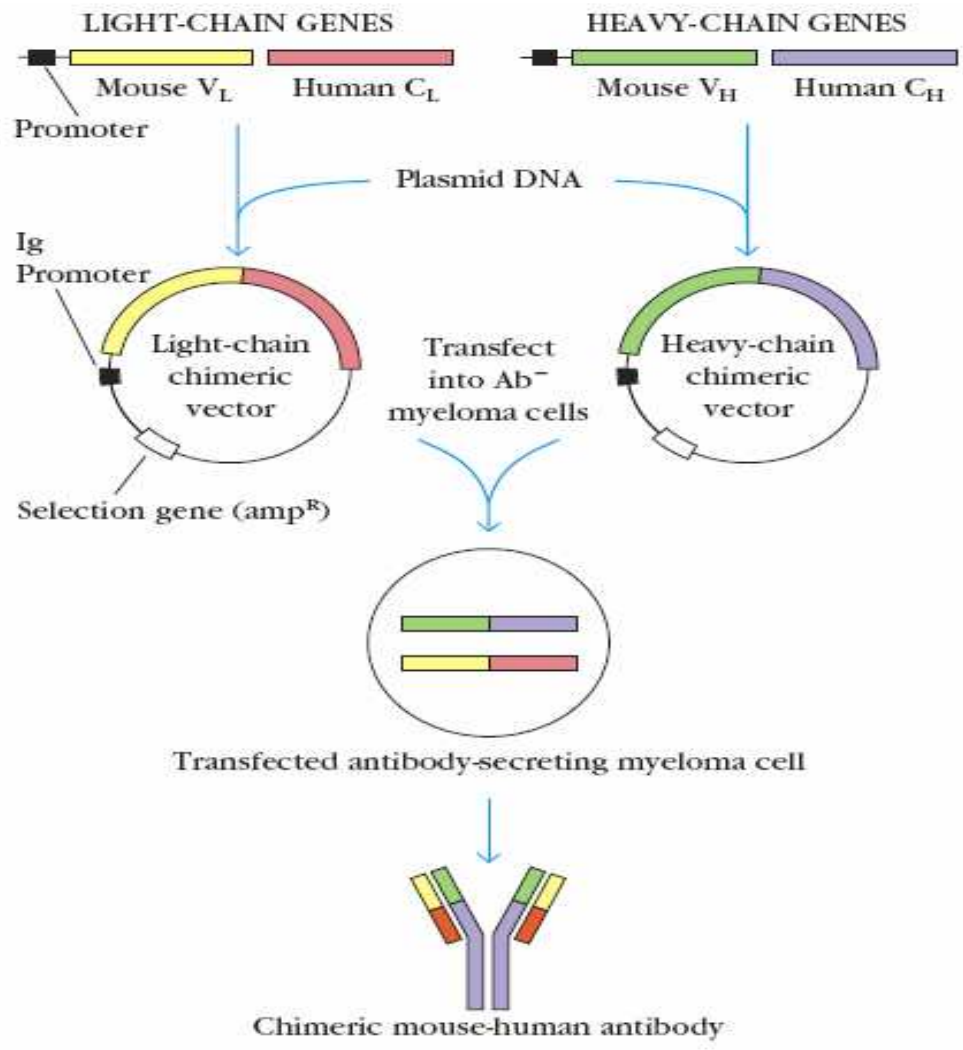

Figure 2.8: Production of chimeric monoclonal antibodies. Monocistronic expression vectors containing mouse variable domains (VH & VL) with human constant domains (CH & CL) are constructed. These expression vectors are transfected into non-antibody secreting myeloma cells and are then cultured in medium containing ampicillin (amp) that allows only the growth of transfectoma cells secreting the chimeric antibody. Humanized mAbs may also be similarly produced using humanized VH and humanized VL instead.

Source: Goldsby et al. 2002.

2.5 ANTI-C2 MONOCLONAL ANTIBODY

The anti-C2 mAb, also known as ior c5, is a mouse IgG_1 generated by the immunization of Balb/c mice with a human colorectal cell line SW1116 (colorectal adenocarcinoma). The splenocytes of the immunized Balb/c mice were fused with SP 2/O Ag 14 nonsecreting cells (Vazquez et al. 1992) and the selected hybrid cells were expended and inoculated into previously primed Balb/c mice for ascitic fluid production. Monoclonal antibodies were purified from mouse ascitic fluid by Protein A-Sepharose affinity chromatography (Vazquez et al. 1993).

This antibody recognizes a tumor associated antigen (TAA) referred to as C2, which is a glycoprotein over-expressed on the surface of colorectal and ovarian cancer cells and does not recognize other antigens such as carcinoembryonic antigen (CEA), Lewis a, Lewis b, or the antigens from the membrane of the mononuclear peripheral cells and red blood cells (Solano et al. 2003).

Immunocytochemical staining also show a strong surface and cytoplasmic reactivity with the colorectal cancer cells lines SW1116 and SW948, but not with various other cell lines: breast carcinoma (MDA-MB-134, 157, 435), melanoma (A-375, M-14, FEM-X), lung carcinoma (U-1752, U2020), B-cell lymphoma (Raji), T-cell leukemia (CEM) as well as peripheral blood mononuclear cells including granulocytes and erythrocytes (Vazquez et al. 1995).

The TAA C2 is a neuraminidase and periodate resistant, pronase and alkali-sensitive epitope present on a major 145 kDa and a minor 190 kDa glycoprotein complex (Ramos-Suzarte et al. 2007). Molecular structure and tissue expression of the TAA C2

is different from previously described colorectal and ovarian TAA because C2-antigen is essentially organ-specific (colon) and free C2-antigen has not been detected in blood of patients with tumors (Ramos-Suzarte et al. 2007). Therefore, TAA C2 gives an advantage as a target for radio-immunodiagnosis of these malignancies over other commonly used TAA, such as CEA, Cancer Antigen (CA)-125, 17-1A, epidermal growth factor receptor (EGF-R), which are all either detected as shed antigens in the serum or expressed in a normal tissue.

It was demonstrated that anti-C2 mAb conjugated with technetium-99m (^{99m}Tc-anti-C2 mAb) recognized colorectal tumors with 94% of sensitivity and 100% of specificity (Ramos et al. 2003). The very limited expression in normal human adult tissues of TAA C2 had also been confirmed using radioimmunoscintigraphy with ^{99m}Tc-anti-C2 mAb for the diagnosis of ovarian cancer (Solano, 2003). Conventional cancer therapies such as chemotherapy and radiotherapy often results in a large-scale destruction of healthy normal cells in addition with destruction of cancerous cells as conventional therapies are not tumor specific. Therefore this mAb which possesses very high specificity against TAA C2 which is not present in normal cells is a highly eligible candidate as a therapeutic alternative for colorectal and ovarian cancer patients (Tejuca et al. 2004).

Nevertheless, anti-C2 mAb being a mouse monoclonal antibody has many major drawbacks such reduced half-life in humans, poor recognition of mouse antibody effector domains by the human immune system and perhaps the, most detrimentally, a significant HAMA response upon administration in human during immunotherapy. Therefore, to overcome the limitations and the drawbacks of this antibody, an engineered form of this antibody is required in order for anti-C2 mAb to be an effective therapeutic agent.