SELECTION OF T CELL RECEPTOR-LIKE ANTIBODY REACTIVE TO *Mycobacterium tuberculosis* 16 kDa HEAT SHOCK PROTEIN FROM A HUMAN ANTIBODY PHAGE DISPLAY LIBRARY

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by

SYLVIA ANNABEL DASS

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To the two love of my life, I dedicate my work to you.

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LIST OF ABBREVIATIONS

ABTS	2,2‘-azino-bis(3-ethylbenzenethiazoline-6-sulphonic acid
ADCC	Antibody-dependent cell-mediated cytotoxicity
APS	Ammonium Persulfate
BCR	B cell receptor
BSA	Bovine serum albumin
C	Constant
CDC	Complement-dependent cytotoxicity
CDRs	Complementary determining regions
C_H	Constant heavy chain
C_H1	Constant heavy chain
C_H2	Constant heavy chain 2 ()
C_H3	Constant heavy chain 3
C_L	Constant light chain
Cond 1	Condition 1
Cond 2	Condition 2
Cond 3	Condition 3
CT	Computed tomography

D	Diversity
DAMPs	Danger associated molecular patterns
DMSO	Dimethyl sulfoxide
dH_2O	Distilled water
DNA	Deoxyribonucleic acid
E.coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
Fab	Fragment antigen binding
Fc	Fragment crystallisable
FDA	Food and Drug Administration
H	Heavy
HAMA	Human anti-mouse antibodies
HLA	Human leukocyte antigen
HRP	Horseradish peroxidase
HSP	Heat shock protein
IB	Inclusion body
IEDB	Immune Epitope Database and Analysis Resource
Ig	Immunoglobulin

IGRAs	Interferon-gamma (IFN-γ) release assays
IMGT	International ImMunoGeneTics information system
IPTG	Isopropyl-β-D-1-thiogalactopyranoside
J	Joining
L	Light
MHC	Major histocompatible complex
mRNA	Messenger ribonucleic acid
Mtb	*Mycobacterium tuberculosis*
MW	Molecular weight
MWCO	Molecular weight cut-off
Native-PAGE	Native-polyacrylamide gel electrophoresis
NK	Natural killer
OD	Optical density
PAMPs	Pathogen-associated molecular patterns
PCR	Polymerase chain reaction
PBS	Phosphate-buffered saline
PEG	Polyethylene glycol
RF	Replicative form
scFv	single chain variable fragment

sdAb	single domain antibody
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide
ssDNA	single-stranded DNA
tascFvs	Tandem single chain variable fragment
TB	Tuberculosis
TCR	T cell receptor
TEMED	Tetramethylethylenediamine
Treg	Regulatory T cells
TST	Tuberculin skin test
UV	Ultraviolet
V	Variable
VH	Variable heavy chain
VL	Variable light chain
WHO	World Health Organization
B2M	β_2-microglobulin

LIST OF SYMBOLS

κ	Kappa
λ	Lambda
γ	Gamma
δ	Delta
ε	Epsilon
ζ	Zeta
η	Eta
α	Alpha
˚C	Degree celsius
%	Percentage
c.f.u	Colony forming unit
kDa	Kilodalton
kV	Kilovolt
mL	Millilitre
mm	Milimeter
mM	Milimolar
mΩ	Miliom
rpm	Revolutions per minute

μg	Microgram
μL	Microliter
μM	micromolar

PENJANAAN ANTIBODI BERFUNGSI SEPERTI RESEPTOR SEL T REAKTIF TERHADAP 16 kDa PROTEIN KEJUTAN HABA (HSP) MENGGUNAKAN PERPUSTAKAAN PAMERAN FAJ ANTIBODI MANUSIA

ABSTRAK

Antibodi berfungsi seperti reseptor sel T juga dikenali sebagai antibodi mimik reseptor sel T telah menarik perhatian ramai penyelidik sejak kebelakangan ini kerana fungsinya yang menggabungkan ciri keistimewaan keimunan humoral and keimunan perantaraan sel dalam satu platform. Ini dimungkinkan oleh perkembagaan teknologi, khususnya kejuruteraan genetik dan pameran faj. Focus utama kajian ini bertumpu kepada penjanaan antibodi mimik reseptor sel T, yang boleh menyumbang kepada pembangunan diagnostik dan terapeutik tuberkulosis pendam kerana beban global tuberkulosispendam terus meningkat. Sebagai sasaran, peptida protein HSP 16 kDa antigen *Mycobacterium tuberculosis* (*Mtb*) yang dipaparkan oleh kompleks histocompatible utama (MHC) HLA-A*2, HLA-A*11 dan HLA-A*24 telah digunakan dalam kajian ini. Protein HSP 16 kDa merupakan calon yang sesuai bagi penyelidikan tuberkulosis pendam kerana ia berfungsi untuk memastikan bakteria tuberkulosis hidup dalam tempoh dorman. Kesemua kompleks peptida antigen 16 kDa MHC telah dihasilkan melalui proses pertukaran peptida berintegrasi sinaran UV. Sebelum itu, kompleks peptida UV-sensitif MHC bagi setiap HLA telah dihasilkan melalui penggabungan rantai berat HLA yang specifik, rantai ringan β2M bersama dengan peptida sensitif-UV. Pendedahan terhadap sinaran UV menyebabkan peptida sensitif-UV berkecai lalu membenarkan pertukaran peptida sasaran antigen 16 kDa

untuk membentuk kompleks peptida sasaran 16 kDa MHC. Teknik panning plat konvensional telah diaplikasikan bagi pemilihan antibodi mimik reseptor sel T dengan menggunakan perpustakaan faj antibodi domain manusia. Perpustakaan antibodi domain manusia yang digunakan dibina berdasarkan segmen rantai berat VH3-23 dengan kepelbagaian sintetik yang diperkenalkan ke wilayah CDR1, CDR2 dan CDR3. Berdasarkan kepada analisis jujukan, setiap sasaran HLA memperolehi sebuah monoklon antibodi mimik reseptor sel T (HLA-A * 2: A2 / Ab; HLA-A * 11: A11 / Ab; HLA-A * 24: A24 / Ab). Selain itu, analisis jujukan juga telah mendedahkan bahawa klon A2 / Ab tergolong dalam IGHV3 V-gen, IGHD3-9 * 01 D-segmen, IGHJ4 * 01 J -segmen; klon A11 / Ab tergolong dalam IGHV3 V-gen, IGHD3-16 * 01 D-segmen, IGHJ4 * 02 J-segmen; dan klon A24 / Ab tergolong dalam IGHV3 V-gen, IGHD2-8 * 02 D-segmen, IGHJ4 * 02 J-segmen. Penghasilan protein monoklon antibodi mimik reseptor sel T A2/Ab, A11/Ab dan A24/Ab berjaya tercapai. Dari segi fungsi, ketiga-tiga protein antibodi bekebolehan untuk berfungsi dalam keadaan larut, di samping dengan memaparkan interaksi yang kuat dengan sasaran antigen 16 kDa masing-masing. Sebagai kesimpulan, penemuan daripada kajian ini dapat diaplikasikan dalam pembangunan dan penambahbaikan diagnostik dan terapeutik tuberkulosis pendam.

SELECTION OF T CELL RECEPTOR-LIKE ANTIBODY REACTIVE TO *Mycobacterium tuberculosis* 16 kDa HEAT SHOCK PROTEIN FROM A HUMAN ANTIBODY PHAGE DISPLAY LIBRARY

ABSTRACT

T cell receptor (TCR)-like antibody has drawn the attention of many researchers recently due to its dual functionality of sandwiching the best of the humoral (antibody) and cell-mediated immunity (T cell) in a single platform. This is possible through the advancement in technology, specifically genetic engineering and phage display. The primary focus of this study was to generate TCR-like antibody for the development of latent tuberculosis diagnostics and therapeutics since the global burden contributed by latent tuberculosis is constantly increasing. As targets, *Mycobacterium Tuberculosis (Mtb)* heat shock protein (HSP) 16kDa antigen peptide presented by the major histocompatible complex (MHC) HLA-A*2, HLA-A*11 and HLA-A*24 were used in this study. The 16 kDa antigen heat shock protein is responsible for the survival of the TB bacilli during dormancy, making the protein a suitable candidate for latent tuberculosis investigation. Target peptide-MHC complexes were generated via a UV-mediated peptide exchange process. Prior to peptide exchange, UV-sensitive photolabile peptide-MHC complex of all three HLAs were formed by refolding each of the HLA heavy and β2M light chains along with a UV-sensitive peptide. Upon UV exposure, the photolabile peptide was cleaved at the UV-sensitive amino acid site allowing the exchange of 16 kDa antigen target peptide to form the 16 kDa target peptide-MHC complex. Conventional plate panning

technique was applied for the selection of TCR-like antibody using a human domain antibody phage library. The human domain antibody library used was constructed based on the VH3-23 heavy chain germ-line segment with synthetic diversity introduced into CDR1, CDR2 and CDR3 regions. For each HLA target, a single TCR-like domain antibody clone (HLA-A*2: A2/Ab; HLA-A*11: A11/Ab; HLA-A*24: A24/Ab) was successfully obtained and confirmed via sequence analysis. The sequence analysis revealed that clone A2/Ab belongs to IGHV3 V-gene, IGHD3-9*01 D-segment, IGHJ4*01 J-segment; clone A11/Ab belongs to IGHV3 V-gene, IGHD3-16*01 D-segment, IGHJ4*02 J-segment; and clone A24/Ab belongs to IGHV3 V-gene, IGHD2-8*02 D-segment, IGHJ4*02 J-segment. In addition to the successful expression and purification of all three clones, the generated A2/Ab, A11/Ab, and A24/Ab TCR-like antibody proteins proved to be functional in soluble form and exhibited strong binding capacity against their respective 16 kDa antigenic targets. In conclusion, the favourable findings of this study serve as a propitious platform for the development and improvement of latent tuberculosis diagnosis and therapeutics.

CHAPTER 1

INTRODUCTION

1.1 Overview of the immune system

The immune system is a diverse defence system that has evolved in order to protect individuals from invading pathogenic microorganism and cancer (Judith et al., 2013). The system is capable of generating a huge diversity of cells and molecules that are able to recognize as well as eliminate a diversity of foreign invaders. It is able to differentiate healthy, unhealthy and infected cells from each other by identifying fine chemical alternations and danger signals called danger associated molecular pattern (DAMPs). It also has the capacity to distinguish between foreign bodies (non-self) and the body's own cells and proteins (self) by recognizing a set of signals emitted by the infectious microbe known as pathogen-associated molecular patterns (PAMPs) (Shefali Khanna Sharma & Naidu, 2016).

In general, the human immune system is divided into innate and adaptive immune system that work hand in hand to counteract a threat as illustrated in **Figure 1.1**. Once a foreign body has been distinguished, the innate immune (non-specific) system acts as a first line of defence. The innate immune system (**Figure 1.1 (a)**) comprises of cells and proteins that are constantly present and prepared to counteract or eliminate threats. Components of the innate immune system include physical epithelial barriers, phagocytic leukocytes, dendritic cells, natural killer (NK) cells and circulating plasma proteins such as lysozyme, interferon, and complement (Wood, 2006c). These soluble proteins contribute significantly to the immune system upon encountering a threat.

The adaptive immune system (**Figure 1.1 (b)**) comes in control in the event pathogens are able to evade and overcome the innate immune defences or if the pathogens persist. Adaptive immune responses are antigen-specific whereby it is capable of recognizing and eliminating specific foreign microorganisms in contrast to the general response of the innate immune system to a microorganism species or cancerous cells (Chaplin, 2010). In other words, the adaptive immune system responds differently to all members of a pathogen species. The adaptive immune system displays significant characteristics, which includes antigenic specificity, diversity in recognition, immunological memory, and recognition of self and non-self (Judith et al., 2013). The major components of adaptive immune response are lymphocytes and antigen-presenting cells. The two key players, B (humoral immunity) and T (cell-mediated immunity) lymphocytes/cells are significantly different in terms of structures, role and mechanism exhibited in an immune response.

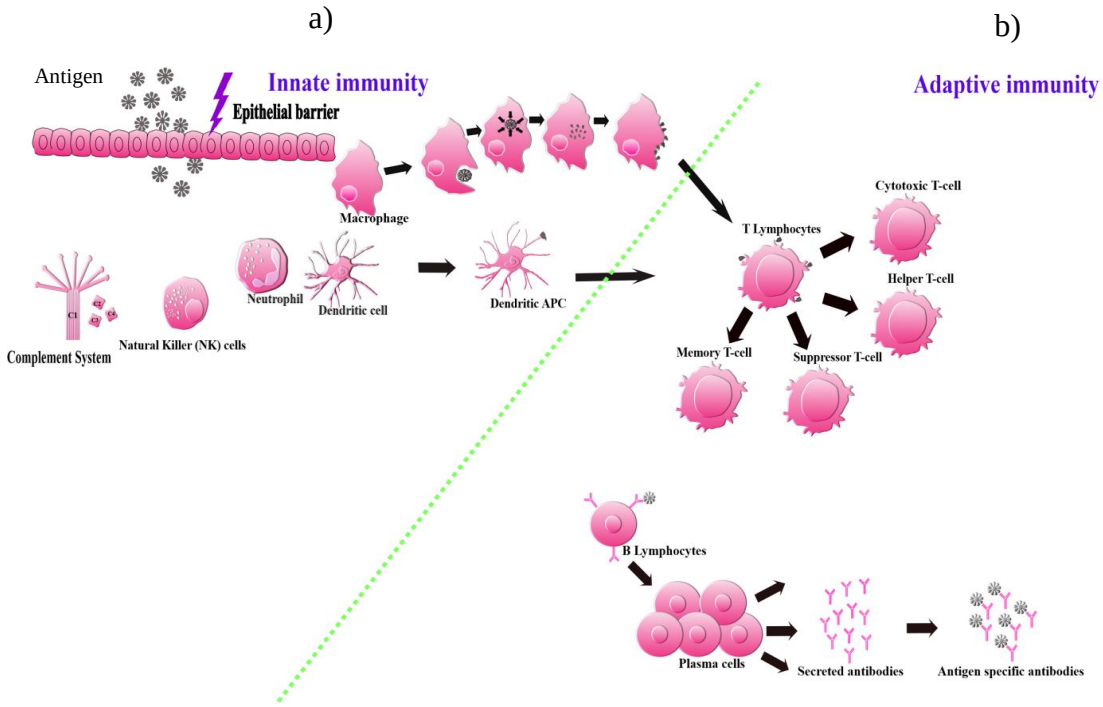

Figure 1.1 Overview of the immune system whereby the innate and adaptive immune system works hand in hand to provide defence. (**a**) Components of the innate immune system act as the first line of defence upon exposure to antigen. The antigen presenting cells such as dendritic cells and macrophages present antigenic peptides for further processing by the adaptive immune components (lymphocytes). (**b**) The adaptive immune responses come in control when pathogens are able to evade or overcome the innate immune defences and to generate memory against encountered pathogens.

1.2 B-cell mediated immunity

Antibodies, known as immunoglobulins (Ig) are glycoproteins secreted by the plasma B cells with its primary role in humoral immune responses. It was initially identified to be residing in the serum by A. Tiselius and E. A.Kabat, in 1939 (Buchacher & Iberer, 2006). In general, antibodies exist as soluble and/or membrane-bound in the immune system (Wood, 2006c). The membrane-bound antibody generated by a naive/immature B cell acts as a B cell receptor (BCR) for antigen recognition(Alberts et al., 2002a). Upon antigen activation (with the aid of helper T cell), the naive B cell undergoes clonal expansion in which cell proliferation and differentiation occurs to form antibody-secreting plasma cells and memory B cell as shown in **Figure 1.2** (J. A. Owen et al., 2013a). Simultaneously, the affinity maturation process of generating antibodies with higher binding affinities towards the encountered antigen takes place (Mishra & Mariuzza, 2018; J. A. Owen et al., 2013a).

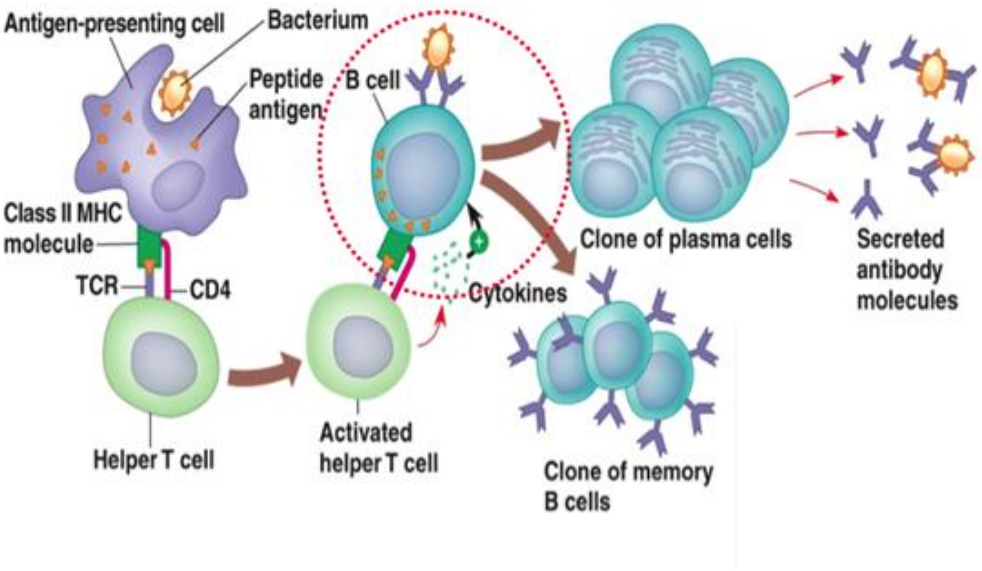

Figure 1.2 Activation of B cell upon antigen recognition. The activated B cell undergoes clonal expansion to generate memory B cells and plasma cells which secrete soluble antibody molecule specific to the antigen (Reece & Campbell, 2008).

1.3 Antibody and its structure

The basic antibody molecule structure is characterized as a Y-shaped structure that consists of two identical heavy (H) (~50 kDa) and light (L) (~25 kDa) peptide chains with disulfide bonds holding each heavy chain to a light chain and at the same time clutches the two pairs together (Wood, 2006c). There are two types of light chain termed as kappa(κ) and lambda (λ) (Esparvarinha et al., 2017). They share similar structures but vary in the genes coded and exist independently from each other in an antibody molecule. The lambda (λ) genes are encoded on chromosome 22 while the kappa (κ) genes are encoded on chromosome 2 (Townsend et al., 2016). It has been reported that the distribution of kappa (60%) light chain antibody in human peripheral blood is higher compared to the lambda (40%) light chains (J. Owen et al., 2007). On the other hand, there are five distinct heavy chains (α, μ, δ, γ and ε) located at the constant region of an antibody which contributes to the antibody isotyping and its respective functions (Alberts et al., 2002a). The antibody structure is further divided into a variable (V) and constant (C) region that covers the top and stem of the Y structure respectively (Wang et al., 2007). The variable region of the light (V_L) and heavy (V_H) chain is made up of approximately 110 amino acid each and is responsible for antigen binding as well as the distinct specificity displayed by the different antibodies while the constant region of the light (C_L) and heavy (C_H) comprises of a rather constant sequence and remains the same between antibodies (Janeway et al., 2001b).

The dissection of the antibody molecule structure via protease papain cleaves the structure into three fragments. The first two identical fragments known as fragment antigen binding (Fab) fragments consist of the entire light chains coupled with the V_H and constant heavy chain 1(C_H1) portions of the heavy chain and are

associated with antigen binding function (Schroeder & Cavacini, 2010). The third fragment termed as fragment crystallizable (Fc) fragments which consist of two identical fragments derived from constant heavy chain 2 (C_H2) and constant heavy chain 3 (C_H3) domains (IgM and IgG consist of second, third and fourth C_H domains), not only are responsible for the isotyping and subclassification of antibodies but also facilitate the effector mechanism of the immune responses (Chaplin, 2010).

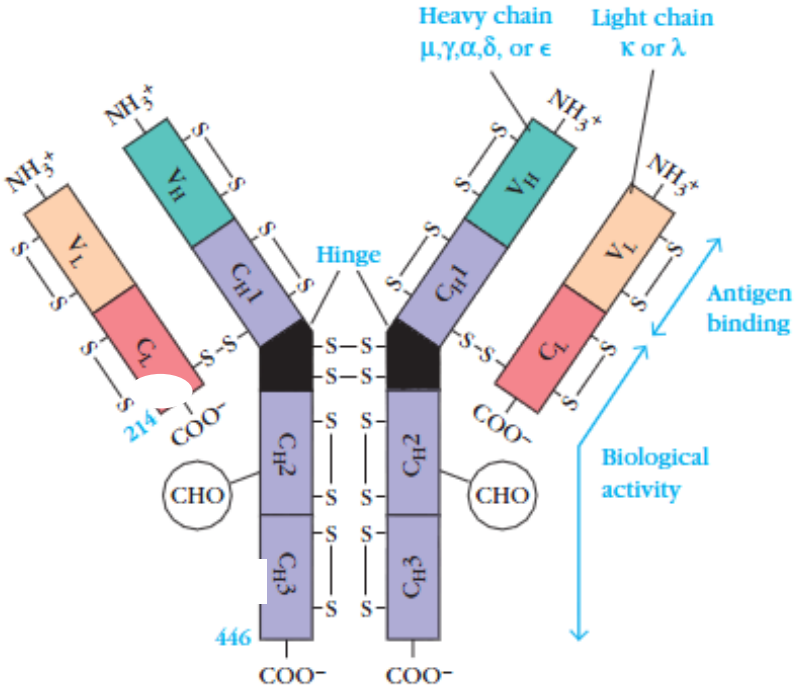

Figure 1.3 Basic structure of an antibody molecule. The antibody comprises two heavy and light chains. Disulfide bonds link the heavy to the light chains and also both heavy chains to each other (J. Owen et al., 2007).

1.4 Antibody isotypes

In general, the antibody mediates several effector functions as part of its role in providing immune defence. Some of the antibody-mediated effector roles include opsonization, activation of the complement system, antibody-dependent cell-mediated cytotoxicity (ADCC) and transcytosis (J. Owen et al., 2007). The various features of the Fc fragment are responsible for these effector mechanisms resulting in the existence of the diverse antibody isotypes which are classified based on the distinct heavy chains acquired (Spiegelberg, 1974). The five isotypes of antibody in human are IgA (α), IgM (μ), Ig D (δ), IgG (γ) and Ig E (ε) as shown in **Figure 1.4** (K. Chen & Cerutti, 2011).

IgG, the most prevailing antibody among all immunoglobulins comprises of two heavy and light chains and is subdivided into four classes (Ig G1, Ig G2, Ig G3, and Ig G4) each singularized based on the gamma (γ) chain sequence variation (Vidarsson et al., 2014). The functionalities of the IgG subclasses are determined by the individual amino acid acquired by each class. One of the prominent roles of IgG in immune defence specifically Ig G1, Ig G2 and Ig G3 (most effective) are as the activators of the complement cascade pathway (Giuntini et al., 2012). Another effector function of IgG is transplacental transport in which Ig G1, Ig G3, and Ig G4 are capable of crossing through placenta ensuring protection for the growing fetus (Palmeira et al., 2012). The IgG subclasses also act as opsonization mediators with Ig G1 and Ig G3 possessing the strongest affinity to Fc receptors compared to Ig G2 and Ig G4 (J. Owen et al., 2007).

Ig M is the preliminary antibodies produced upon initial exposure to an antigen which exists as a pentamer of five Ig M molecules bridged together via

disulfide bonds and polypeptide joining (J) chains giving a total of ten antigen-binding sites (Schroeder & Cavacini, 2010). The pentameric structure of Ig M contributes eminently to its roles as well as the high capability of antigen binding when compared to other Ig molecules. The prominent functions of Ig M include activating the complement pathway, neutralizing bacterial and viral infection, complementing IgG responses and also act as a secretory antibody (Boes, 2000).

Ig D was initially discovered when a tested myeloma protein failed to respond to the then available antisera of Ig A, Ig M, and IgG which resulted in the further analysis that substantiated its existence (Black, 1997). Ig D is found to be expressed along with Ig M by the mature B cells (Rogers et al., 2006). Structurally, Ig D possesses a standard Ig molecular complex and is detected at a minimal level in serum concentration (Janeway et al., 2001b). The functions of Ig D were poorly understood initially although later studies highlighted Ig D's immune functions that included enhancement of the mucosal immunity, activation of several innate cells such as basophils and mast cells and stimulation of proinflammatory roles (K. Chen & Cerutti, 2011).

Ig A is the most predominant secretory immunoglobulin found in secretions like maternal milk, tears, saliva, and mucus (Woof & Kerr, 2006). Basically, Ig A exists as monomers although it may form polymers with all forms acquiring a J-chain polypeptide (Suzuki et al., 2015). The two subdivision of Ig A, IgA1 and IgA2 are differentiated based on the length of the respective hinge regions (Wood, 2006c). In terms of function, Ig A provides protection against virus and bacteria by prohibiting pathogen attachment to the mucosal cells. Another prominent function of Ig A is to deliver neonatal immunity (passive immunity) through breast milk which highlights the importance of breastfeeding.

The existence of Ig E was first evidenced by Prausnitz and Kustner who later authenticated Ig E's role in type 1 hypersensitivity reactions (Hamilton, 2005). Having structural similarity to a basic Ig structure, Ig E is present at a very low level in human sera. Ig E is found to be responsible for arbitrating the instantaneous hypersensitivity reactions precisely by activating mast and basophil cells and antigen presentations in addition to its counter to parasitic worm infection (C. E. Owen, 2007) (C. E. Owen, 2007; Platts-Mills, 2001) (Schroeder & Cavacini, 2010).

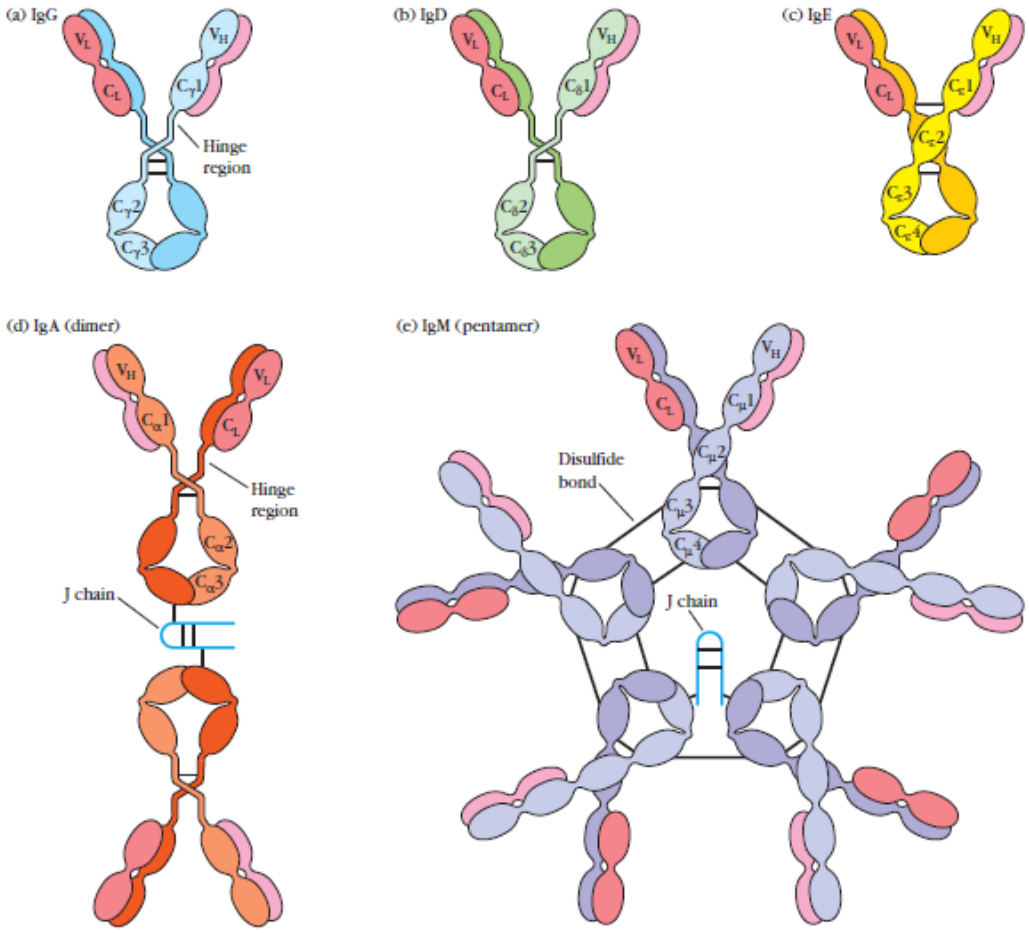

Figure 1.4 A representation of the five antibody isotypes (J. Owen et al., 2007).

1.5 Mechanism of action of antibody

An antibody plays multiple roles in mediating immune responses including neutralization, agglutination, opsonisation, activation of complement and antibody-dependent cell-mediated cytotoxicity. The mechanism of pathogen neutralization varies according to the pathogen. The antibody neutralizes toxin by binding to the toxin and hampering its effects, neutralizes virus by binding to the viral receptor to prevent viral attachment to the host cell and lastly inhibiting the metabolism of bacteria by interfering the adherence of bacteria to the host cells (Forthal, 2014). In terms of agglutination, the antibody confines the pathogens in clumps which prevent the escape of pathogens from the infection sites for elimination by phagocytosis (Roche et al., 2015). Another prominent function of the antibody is opsonisation whereby the dual binding of the antibody molecule to the pathogen through its Fab region and the phagocyte receptors via Fc portion respectively, induces the phagocytosis of the pathogen (Hart et al., 2004). Activation of the complement system is another important role of an antibody (IgM and some of the IgG subclasses only) which leads to the complement-dependent cell cytotoxicity (Charles A Janeway & 2001). The complement system consists of a cascade of interconnected proteins which upon activation, contribute to the immune defence through inflammation, chemotaxis of eosinophils, neutrophils and monocytes, and activation of mast cell and neutrophils (Wood, 2006a). The final and essential role of antibody is to mediate antibody-dependent cell-mediated cytotoxicity (ADCC). This is possible due to once again the dual binding features of the antibody of binding to the infected target cells (Fab region) and cells such as neutrophils, macrophages and natural killer cells (Fc part), resulting in the elimination of the infected cells (J. Owen et al., 2007).

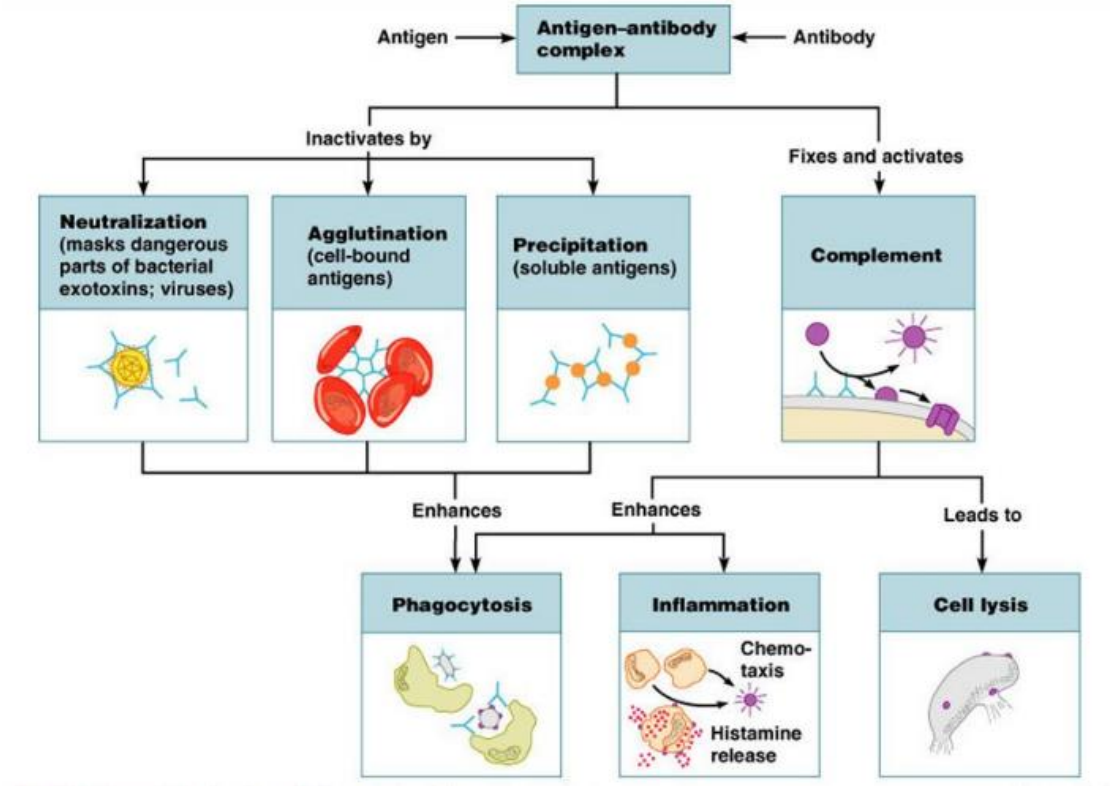

Figure 1.5 The various mechanism of action of the antibody (Cummings, 2004)

1.6 Cell-mediated immunity

Cell-mediated immunity plays a fundamental role in the immune defence and the T lymphocyte/cell is the ace behind it. The generation of T cells begins in the bone marrow similar to B cells followed by maturation in the thymus prior to the circulation of the mature T cells in the blood and lymphoid tissues(Ochoa & Makarenkova, 2005). The helper and cytotoxic T cells are distinguished based on distinctive cell surface molecules expressed separately on the two subsets of T cells that are directly involved in cell-mediated immune responses (LaRosa & Orange, 2008).

Antigen recognition by both helper and cytotoxic T cells are possible through the actions of T cell receptor (TCR) which distinguishes foreign/antigenic peptides displayed on cell surface glycoproteins known as major histocompatible complex (MHC) molecules (Janeway et al., 2001a). Structurally, TCR is a heterodimer that comprises of an α and β glycoprotein chain linked via disulfide bond with each chain acquiring a constant and variable domain (**Figure 1.6**) (Wood, 2006d). The capability of TCR in recognizing a wide range of antigenic peptide-MHC is contributed largely by the variable domain which is generated from diversification of the variable (V), diversity (D) and joining (J) gene segments (Huang et al., 2012). The TCR is also associated with a group of signalling proteins known as CD3 proteins, subdivided into gamma (γ), delta (δ), epsilon (ε), zeta (ζ) and eta (η) that are responsible for activating the intracellular signaling pathway upon antigen recognition by TCR (Choudhuri et al., 2005; Clevers et al., 1988).

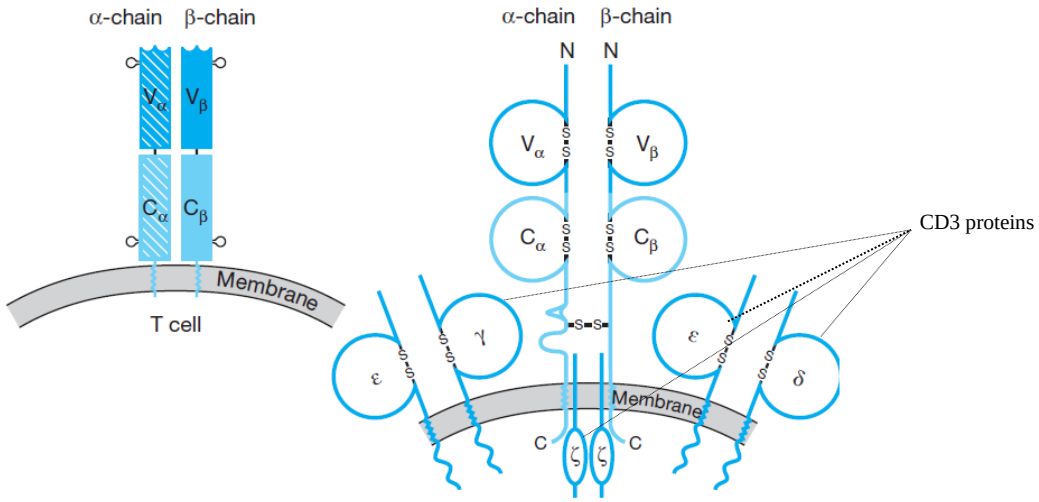

Figure 1.6 Typical structure of the T cell receptor (TCR). The T cell receptor is made up of an alpha and beta glycoprotein chains coupled with a group of CD3 intracellular signalling protein (Wood, 2006d).

1.7 Mechanism of action of T cells

The three subpopulations of T cells, cytotoxic T cells (CD8 T cells), helper T cells (CD4 T cells) and regulatory T cell (Treg) play exclusive roles as part of the immune defence. As the name implies, cytotoxic T cells are responsible in eliminating infected cells upon recognizing the antigenic peptide presented by the MHC class I of the cells (M. H. Andersen et al., 2006). The two different elimination mechanisms exhibited by the cytotoxic T cells are granule exocytosis which involves proteins such as perforin and granzymes found in the granules of the cytotoxic T cell as well as the Fas pathway (Henkart & Sitkovsky, 1994). The helper T cells recognize antigenic peptides presented on the MHC class II molecule of antigen presenting cells and differentiates into two subclasses (T_H1 and T_H2) that determine its function (Wood, 2006b). The T_H1 cells secrete cytokines such as interferon-gamma (IFN- γ) and tumour necrosis factor-alpha (TNF-α), which activates the macrophages to eliminate phagocytosed pathogens, activates cytotoxic T cells and stimulate B cells for antibody secretion targeting intracellular microbes(Wan, 2010). On the other hand, the T_H2 cells secrete interleukins and are associated with defense against the extracellular pathogen, mucosal immunity in the lungs and B cell stimulation for antibody secretion(Alberts et al., 2002b). Contrary to cytotoxic and helper T cells, the regulatory T cells are associated with immune suppression in order to maintain the homeostasis and self-tolerance of the immune system (Kondelková et al., 2010).

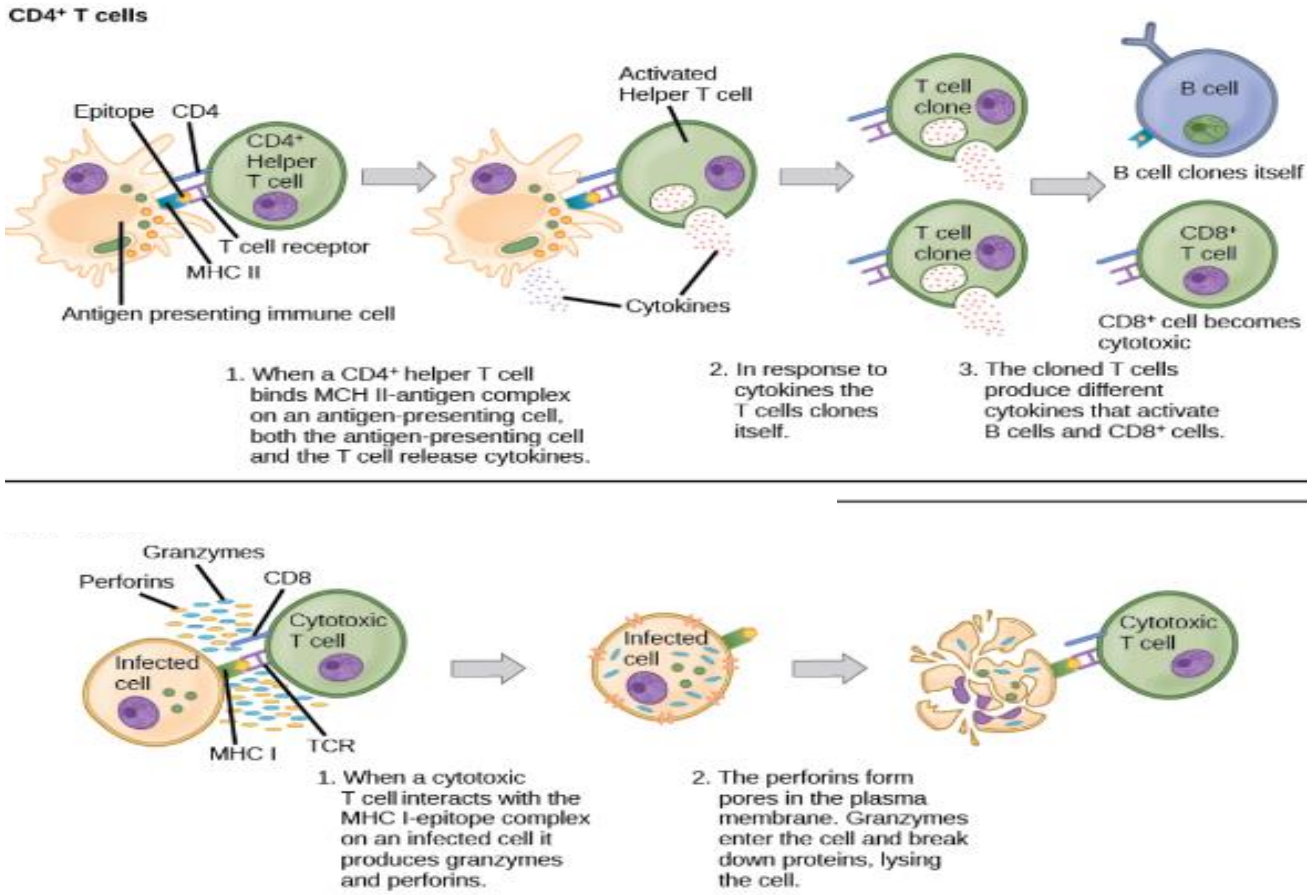

Figure 1.7 A representation of the mechanism of action by the cytotoxic and helper T cells (Molnar & Gair, 2013).

1.8 Major histocompatible complex (MHC) and antigen recognition

Antigen recognition by T cells differs from an antibody in a way that T cells specifically distinguish antigenic peptides presented by the MHC molecules and do not recognize free antigens. As such, the essential role of MHC molecules in cell-mediated immunity is undeniable. In general, MHC molecules are also known as human leukocyte antigen (HLA). In human, MHC molecules are highly diversified glycoproteins encoded by the MHC genes (Alberts et al., 2002c). Class I and class II are the two main classes of MHC molecules involved in T cell's antigen recognition (Wieczorek et al., 2017). In terms of its structure, the MHC class I molecule consists of a lengthier heavy alpha (α) chain (45 kDa) linked to the β_2-microglobulin light chain (12 kDa) and are expressed on the surface of all nucleated cells while the MHC class II molecule which comprises of an alpha chain (30-35 kDa) and beta chain (26-30 kDa) is predominantly expressed on antigen presenting cells such as dendritic cells, macrophages, and B cells (Williams et al., 2002) (Wood, 2006d). In human, there are three loci for each MHC class termed as HLA-A-A, HLA-A-B, HLA-A-C for MHC class I and HLA-DP, HLA-DQ, HLA-DR for MHC class II (Wieczorek et al., 2017).

The T cell subsets are associated with different MHC molecules (**Figure 1.8**). The MHC class I molecule contributes largely in maintaining internal immunosurveillance by displaying antigenic peptides derived from infected or mutated cells to cytotoxic T cells resulting in the elimination of the infected cells (Rock et al., 2016). On the other hand, MHC class II molecules present foreign peptides to helper T cell which transmits intracellular signals that help as its name suggest, to initiate B cells for antibody production and secretion, macrophages for phagocytosis and cytotoxic T cells to eradicate the infected cells (Alberts et al., 2002).

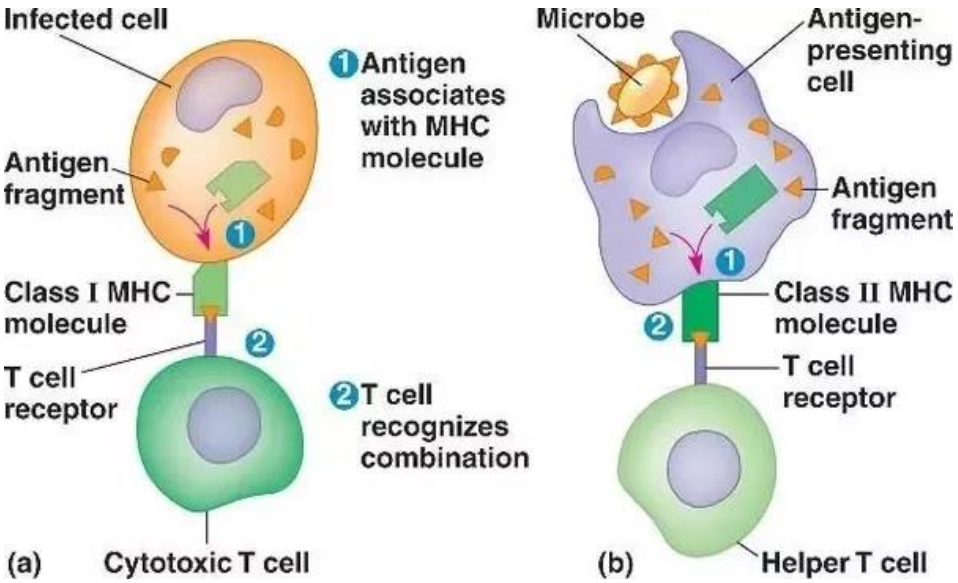

Figure 1.8 Antigenic peptide presentation by MHC class I and II to the respective T cell subsets (Reece & Campbell, 2008).

1.9 Antibody phage display technology

The need for antibodies is constantly increasing as antibodies play a crucial role in the diagnostic and immunotherapy of numerous diseases. Traditionally, antibodies were generated by vaccinating animal model such as mouse, with the target antigen followed by extraction of antibodies from the blood serum. However, the generated antibodies were generally of poor quality and quantity (Dimitrov, 2010). This led to the discovery of other alternative methods including hybridoma and phage display technology.

The pioneer breakthrough of the production of monoclonal antibodies via hybridoma technology in 1975 opened the door for the application of antibody-based therapy in many diseases including infectious diseases and cancer (Freysd'ottir, 2000). To this date, there are approximately forty-seven monoclonal antibody products approved by the Food and Drug Administration (FDA), USA for the treatment of various diseases (Ecker et al., 2015). The hybridoma technology begins with the immunization of a specific organism with the antigen, harvesting the B lymphocytes upon expected immune response before fusing the cells with an immortal myeloma cell line in order to generate the hybridoma (antibody producing) cells and finally the cells undergo a final selection process to obtain the desired monoclonal antibody (Hanack et al., 2016). Although the technology provided a fundamental platform for monoclonal antibody generation, the method also drew several setbacks including being laborious, generated antibodies that were influenced by the immune system of the animal model and immunogenicity through human anti-mouse antibodies (HAMA) response (Liu, 2014).

The evolution of generating monoclonal antibody from hybridoma to phage display technology occurred in 1985 through the discovery of Dr George Smith (Bazan et al., 2012). Based on the understanding that phage genotype and phenotype are physically linked, phage display technology involves the cloning of the desired/target gene into a vector fused with the PIII coat protein gene of a filamentous bacteriophage. Utilizing *E.coli* cells as host, propagation occurs with the generated phagemid displaying the target genes and further in vitro manipulation enables the production of the desired soluble proteins applicable for diagnostics and therapeutics (Dantas-Barbosa et al., 2012) (Liu, 2014).

Antibody phage display technology is the in vitro selection of monoclonal antibody using a high diversity antibody phage display library which consists of bacteriophages displaying antibody fragments of a certain format followed by several rounds of antigen-based selection (Hammers & Stanley, 2014). In comparison to hybridoma technology, antibody phage display technology serves several plus points which include rapid and large-scale antibody generation, flexible to automated selection and screening, enhanced tissue penetration due to small antibody fragment format and reduced immunogenicity as immunization is not required (Bradbury et al., 2011). As such, there is a drastic increase in the number of phage display-derived antibodies in clinical trials after the successful generation of the first Food and Drug Administration (FDA) approved phage display antibody, Adalimumab (Dantas-Barbosa et al., 2012; Frenzel et al., 2016).

1.9.1 M13 bacteriophage

The M13 filamentous bacteriophage is one of the most commonly used phage in antibody phage display system with a basic viral structure of single-stranded viral DNA and protein capsid and known to infect gram-negative bacteria such as *Escherichia coli* (*E. coli*) (**Figure 1.9**) (Bazan et al., 2012). The phage is approximately 900 to 2000 nm in length and 7 nm wide, enveloping the entire DNA genome of 11 genes in a long cylinder (Khalil et al., 2007). A breakdown of the eleven M13 genes function revealed that gene I, IV and XI are for phage assembly, gene II and X for DNA replication, gene III, VI, VII and IX for minor capsid protein, gene VII for major capsid protein and finally gene V for single-stranded (ss) DNA binding (Webster, 1996).

The life cycle of M13 phage begins with the fusion of phage to the bacterium via interaction between the N2 domain of the pIII minor protein capsid and the F pilus tip of the bacterium which mediates the binding of pIII-N1 domain to surface co-receptor TolA protein of the *E.coli* resulting in the depolymerisation of the protein coat and subsequent transfer of the phage single-stranded DNA (ssDNA) into the host (Click & Webster, 1997). The ssDNA is supplemented with a complementary DNA strand by the host to form double-stranded DNA phage chromosome termed as replicative form (RF) (Rakonjac et al., 2017). Phage assembly takes place at the bacterial envelope and the newly assembled phage is secreted into the media (Ledsgaard et al., 2018). The overview of the M13 phage life cycle is shown in **Figure 1.10**.

M13 bacteriophage-based phage display system delivers several benefits to the antibody screening process. Antibody libraries generated from this phage system are

easily manageable throughout the biopanning process as the M13 is non-lytic in addition to the high stability, tolerance to high temperature and extreme conditions acquired by the phage which further proves the capability and suitability of M13 phage (Shim, 2017).