BS4019: Discovery and Development of Therapeutic Antibodies

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Preface

About BS4019

Part I

PART 1 : LECTURES

1 Introduction to Antibodies

This chapter covers rudimentary information on antibodies, including but not limited to the kinds found in the human body, their response to pathogens, their interactions, and their structure.

1.1 The Immune System

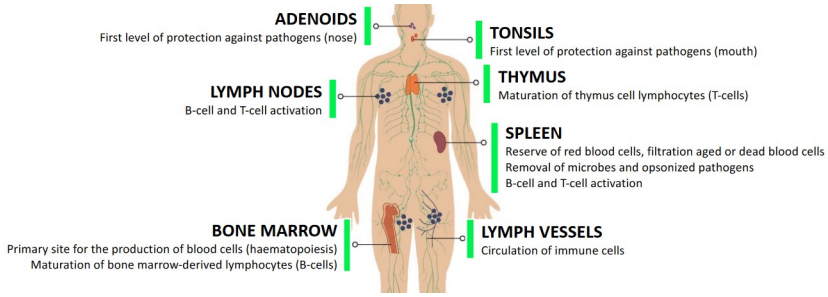

Figure 1.1: Various Organs of the Human Immune System

The human body has numerous tissues and organs that are included in its immune system (i.e., a system that helps fend off pathogens):

1. **Adenoids**

   This is the first level of protection against pathogens in the nose.

2. **Lymph Nodes**

   These enable B and T-cell activations

3. **Bone Marrow**

   This is the site where blood is produced (i.e., haematopoiesis). B-cells also develop here via bone marrow-derived lymphocytes.

4. **Tonsils**

   This is the first level of protection against pathogens in the mouth.

5. **Thymus**

   This organ helps T-cells to mature.

6. **Spleen**

   This acts as a reserve of red blood cells (and also helps filter them). Microbes, opsonized pathogens, and aged or dead red blood cells are also filtered out here.

   B and T-cell activation also happens here.

7. **Lymph Vessels**

   Immune cells are circulated around the body via these.

1.1.1 Innate and Adaptive Immunity

The **innate** immune system enables "non-self" antigens (e.g., pathogens) to be quickly eliminated. Cells in this system present antigens to activate T-cells (hence supporting antibody response).

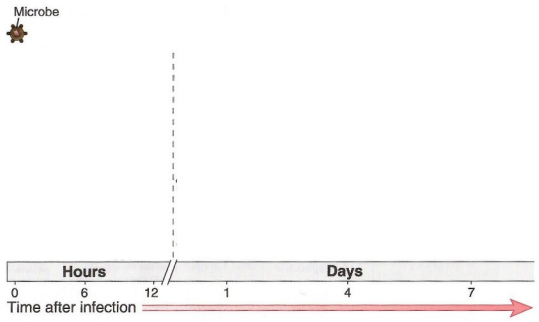

Figure 1.2: Timeline of Infection

The **adaptive** immune system has a slow response time (i.e., after the dashed vertical line above) and improves over time. Only via "memory" does this system quickly respond to known antigens.

1.1.2 Main Cells of the Immune System

The immune system has many cells, of which include:

1. **Macrophages**

   These belong to the *innate* immune system and perform phagocytosis.

   These are antigen-presenting cells.

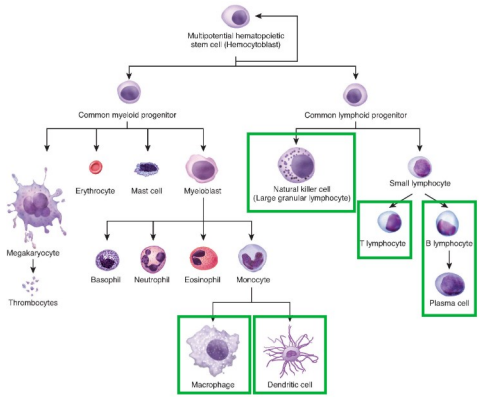

Figure 1.3: Cells of the Immune System

2. **Dendritic Cells**

   These also belong to the *innate* immune system and also play a role in phagocytosis, proteolysis, and the presentation of antigens.

   These cells also play a role in T-cell activation.

3. **Natural Killer Cells**

   These belong to the *innate* immune system. They kill infected or cancer cells.

4. **T-Cells**

   These belong to the *adaptive* immune system; they are also specialized in recognizing non-self antigens via T-cell receptors.

   There are numerous T-cells with different functions.

5. **B-Cells and Plasma Cells**

   These are part of the *adaptive* immune system and play a role in the production of antibodies.

1.1.3 T-Cell Differentiation

T-cells can differentiate into one of four kinds of T-cells:

1. **CD8+ "Cytotoxic" T-Cells**

   These kill cells that display a non-self antigen (e.g., an infected / tumor cell).

2. **CD4+ "Helper" T-Cells**

   These help activate CD8+ T-Cells and also B-Cells.

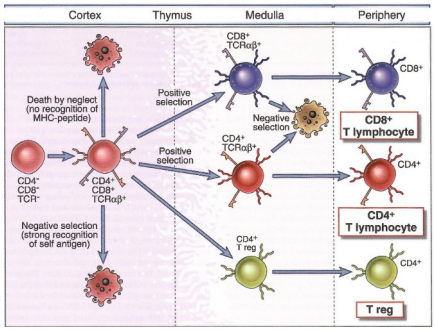

Figure 1.4: Possible T-Cells from Differentiation

3. **CD4+ Regulatory Cells (Treg)**

   These help down-regulate the immune response.

4. **Memory T-Cells**

   A small portion of T-cells go onto become involved in long-term immune responses.

1.1.4 B-Cell Differentiation

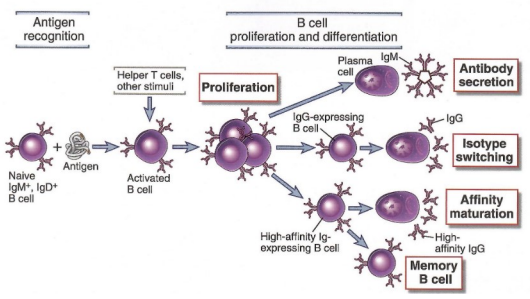

Figure 1.5: B-Cell Differentiation in the Human Immune System

Similarly, B-cells can also go onto mature into one of several different kinds of B-cells:

1. **Naive B-Cells**

   These are B-cells that display antibodies against different kind of antigens' surfaces (with about 10^7 to 10^8 different kinds of specific surfaces).

2. **Activated B-Cells**

   This happens when a naive B-cell binds to a specific antigen. This antigen (see above picture) is then displayed on its surface to help recruit CD4+ T-cells.

3. **Plasma B-Cells**

   These are antibody-producing cells.

   IgM - antibodies with a weak affinity and specificity - are produced and secreted. **IgG** - antibodies with a higher affinity and specificity - are generated in the long run.

4. **Memory B-Cells**

   These are involved in the long-term immune response to previously-encountered antigens.

IgG-secreting antibodies can also be selected for further differentiation to produce higher-affinity IgGs via a maturation process.

1.2 Immune System Responses

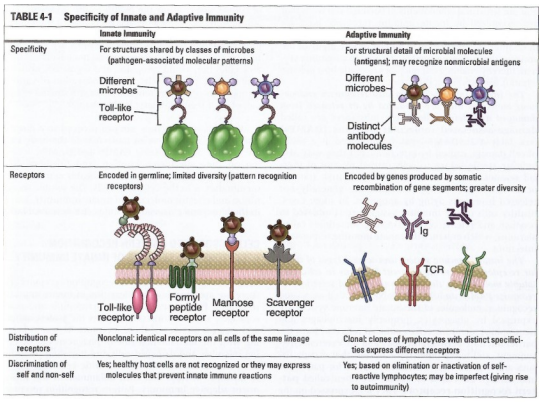

Figure 1.6: Structures Involved in Innate and Adaptive Immunity

The human body's innate immune system relies on patterns that are associated with pathogens and cell damage.

The adaptive immune system relies on specialized molecules with high specificities: **T**-**c**ell **r**eceptors (i.e, **TCR**s) and antibodies.

1.2.1 Antigen-Recognizing Molecules of the Immune System

BS4019 covers a few:

1. **MHC molecules**

   These molecules shows linear peptides on antigen-presenting, infected, or cancerous cells.

TABLE 5-1 Features of Antigen Binding by the Antigen-Recognizing Molecules of the Immune System

Feature	Antigen-Binding Molecule		
	Immunoglobulin (Ig) 	T cell receptor (TCR)* 	MHC molecules* 
Antigen-binding site	Made up of three CDRs in V_H and three CDRs in V_L domains	Made up of three CDRs in V_α and three CDRs in V_β domains	Peptide-binding cleft made of α1 and α2 domains (class I MHC) and α1 and β1 domains (class II MHC)
Nature of antigen that may be bound	Macromolecules (proteins, lipids, polysaccharides) and small chemicals	Peptide-MHC complexes	Peptides
Nature of antigenic determinants recognized	Linear and conformational determinants of various macromolecules and chemicals	Linear determinants of peptides; only 2 or 3 amino acid residues of a peptide bound to an MHC molecule	Linear determinants of peptides; only some amino acid residues of a peptide
Affinity of antigen binding	K_d 10^{-7}–10^{-11} M; average affinity of Igs increases during immune response	K_d 10^{-5}–10^{-7} M	K_d 10^{-6}–10^{-9} M; extremely stable binding
On-rate and off-rate	Rapid on-rate, variable off-rate	Slow on-rate, slow off-rate	Slow on-rate, very slow off-rate

CDR, complementarity-determining region; K_d, dissociation constant; *MHC*, major histocompatibility complex; (only class II molecules depicted); V_H, variable domain of heavy chain Ig; V_L, variable domain of light chain Ig.

Figure 1.7: Some Antigen-Recognizing Molecules

2. **T-Cell Receptors**

   These are receptors that are displayed by T-cells.

   These receptors also help recognize linear peptides that are shown by MHC molecules.

3. **Immunoglobins (i.e., Ig / antibodies)**

   These are secreted by β-cells. Immunoglobins also recognize epitopes of various natures (e.g., proteins, lipids, sugars, etc).

1.2.2 Phases of the Adaptive Immune System

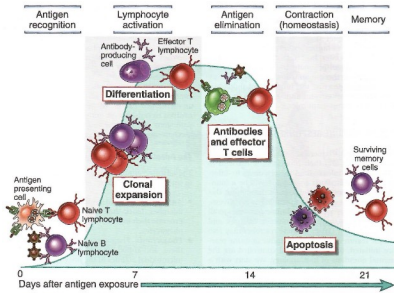

Figure 1.8: Activation of the Adaptive Immune System

The above figure goes in the following order:

1. **Antigen Recognition**

   Antigen-presenting cells (e.g., dendritic cells) show an antigen that is recognized by a naive T cell and / or a naive B-cell recognizes an antigen via an antibody on its surface.

2. **Lymphocyte Activation**

   The specific T-cell is activated and undergoes clonal expansion. The T-cell then differentiates into effector T-cells.

   The specific B-cell becomes activated, undergoes clonal expansion, and differentiates into antibody-producing cells.

3. **Antigen Elimination**

   Cytotoxic T-cells help eliminate infected cells.

   Antibodies also block pathogens and recruit innate immune cells (e.g., NK cells) to eliminate pathogens.

4. **Contraction**

   After pathogens are eliminated, cytotoxic T-cells and antibody-producing B-cells undergo apoptosis (i.e., they kill themselves).

5. **Memory**

   Memory B and T-cells form - these survive into the long term and rapidly produce antibodies in the case of re-infection.

1.2.2.1 Primary and Secondary Responses to an Infection

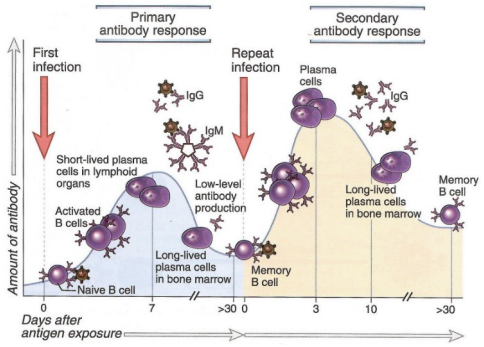

Figure 1.9: Amount of Antibodies over Time

The first response is IgM-rich - because of this, it is relatively weak and non-specific.

The secondary response is IgG-rich - it is stronger and more specific.

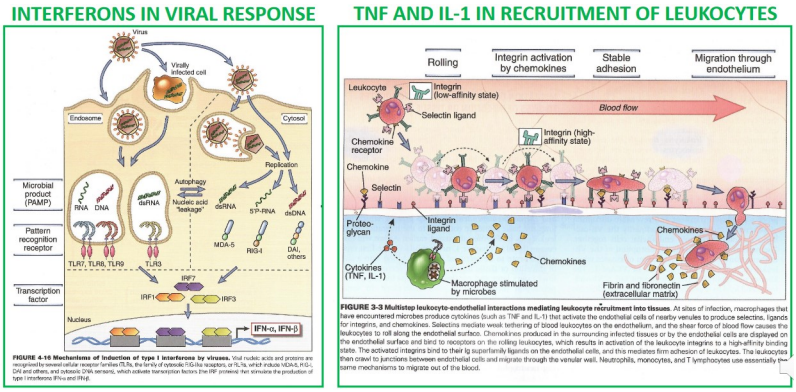

Figure 1.10: Examples of Cytokines in Various Scenarios

1.2.2.2 What are Cytokines?

Cytokines are cell signalling molecules that are involved in the innate and adaptive immune systems.

1.3 Parts of an Antibody

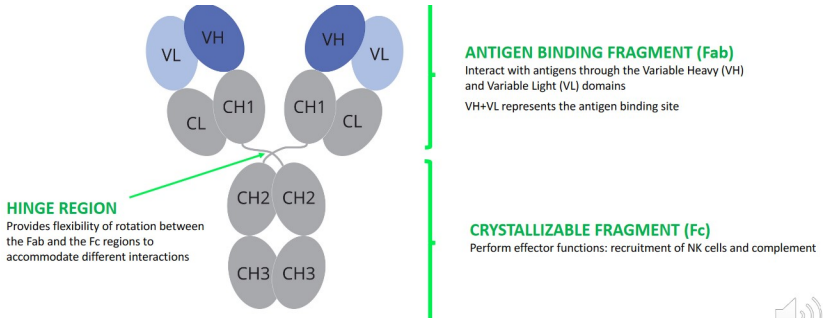

Figure 1.11: Basic Structure of an Antibody

An **antibody** is a protein that is comprised of antigen-binding and crystallizable fragments.

The **antigen binding fragments (Fab)** interact with antigens via **v**ariable **h**eavy (i.e., **VH**) and **v**ariable **l**ight (i.e., **VL**) domains. Together, The VH and the VL form the antigen binding site.

The **crystallizable fragment (Fc)** perform effector functions - they help recruit NK and complimentary cells.

The **hinge region** allows the Fab and Fc regions to rotate and accommodate different interactions.

1.3.1 Light and Heavy Chains

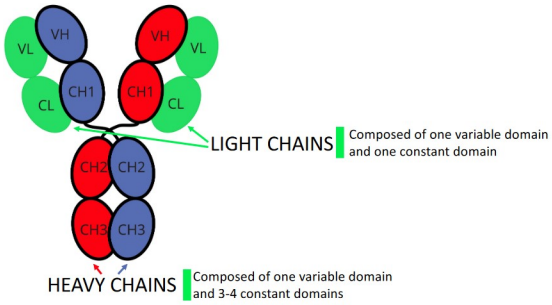

Figure 1.12: Light and Heavy Chains of an Antibody

Light chains have one constant and one variable domain.

Heavy chains have one variable domain and three to four constant domains.

1.3.2 Intermolecular and Intramolecular Disulfide Bonds

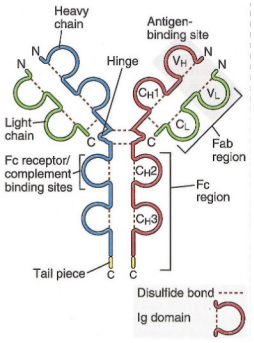

Figure 1.13: Bonds in an Antibody

Antibodies are stabliized by inter- and intramolecular disulfide bonds at the following locations:

1. **Intra-Domain Disulfide Bonds**

   There is one disulfide bond per domain - this contributes to domain stability and fold.

2. **Ch1 - CL Disulfide Bonds**

   There is one of such bond per Fab. This bond stabilizes the heterodimer between heavy and light chains.

3. **Hinge Region Disulfide Bonds**

   There are a variable number of these bonds (depending on the antibody in question).

   These bonds stabilize IgG dimers.

1.3.3 Antibody Isotypes

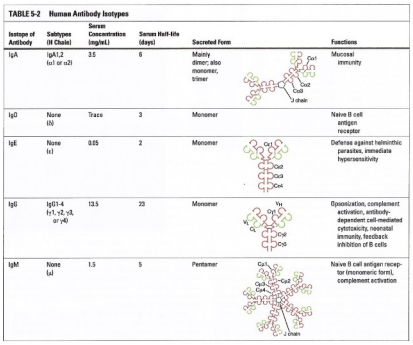

TABLE 5-2 Human Antibody Isotypes

Isotype of Antibody	Subtypes (H Chain)	Serum Concentration (mg/mL)	Serum Half-life (days)	Secreted Form	Functions
IgA	IgA1,2 (α1 or α2)	3.5	6	Mainly dimer; also monomer, trimer	Mucosal immunity
IgD	None (δ)	Trace	3	Monomer	Naive B cell antigen receptor
IgE	None (ϵ)	0.05	2	Monomer	Defense against helminthic parasites, immediate hypersensitivity
IgG	IgG1-4 (γ1, γ2, γ3, or γ4)	13.5	23	Monomer	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neonatal immunity, feedback inhibition of B cells
IgM	None (μ)	1.5	5	Pentamer	Naive B cell antigen receptor (monomeric form), complement activation

Figure 1.14: Various Antibodies Found in the Human Body

The above table shows the various antibodies that are found in the human body.

IgG antibodies are the preferred format for developing antibodies - these have a fast response time to pathogens, have a high affinity, and a long serum half-life.

IgM antibodies are produced in the early phases of an immune reaction (to pathogens) - these antibodies have weak affinities (which are compensated by a pentameric format). However, they can recruit a complement system.

1.4 Antibody-Antigen Interactions

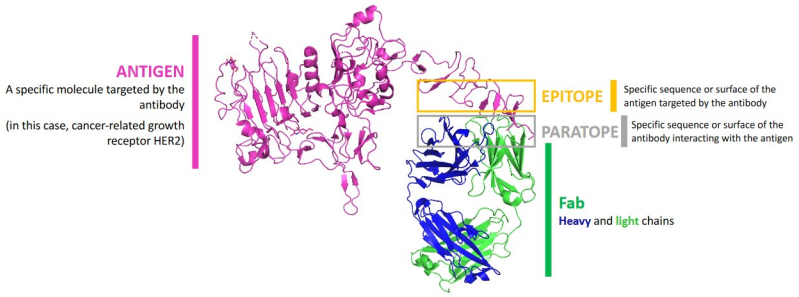

Figure 1.15: Structure of Antibody Labelled

BS4019 uses the following terms:

1. **Antigen**

   This is a specific molecule that is targeted by an antibody.

2. **Epitope**

   This is the specific sequence or surface of an antigen that is targeted by an antibody.

3. **Paratope**

   This is the specific sequence or surface of the *antibody* that interacts with the antigen.

4. **Fab**

   These are made out of heavy *and* light chains.

1.4.1 Complimentary Determining Regions (i.e., CRDs)

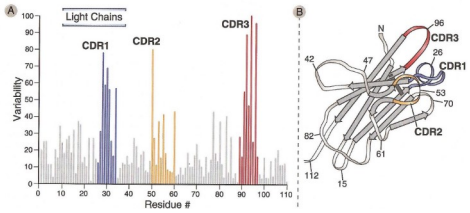

Figure 1.16: CRDs in an Antibody

All VH and VL domains carry three CDRs each - each of these CDRs also vary in sequence composition and length.

The CDRs are hypervariable regions that provide specificity.

2 Therapeutic Antibody Design

This week's (i.e., week 2) lecture focuses on the following topics:

1. Antibody effector functions
2. IgG subclasses and their effector functions
3. Approaches to making therapeutic antibodies
4. Understanding disease biology
5. Therapeutic antibody design

2.1 Surfaces of the Crystallizable Fragment (i.e., Fc)

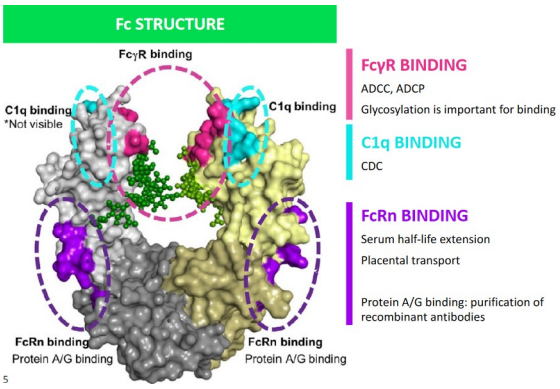

Figure 2.1: Surfaces of the Fc

Different surfaces of the Fc each perform a different function:

1. **FcγR Binding**

   Here, glycosylation is important for binding. This is also the site of ADCC[1] and ADCP[2].

2. **C1q Binding**

   D.

[1]This stands for Antibody-dependent cellular cytotoxicity
[2]This stands for Antibody-Dependent Cellular Phagocytosis

3. **FcRn Binding**

   Protein A/G binding allows for the purification of recombinant antibodies.

   This receptor is also responsible for placental transport and serum half-life extension (see below).

2.1.1 Complement System Recruitment

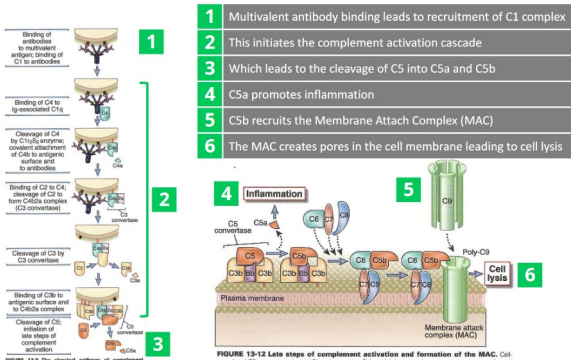

Figure 2.2: Recruitment Process of the CDC

There are six main steps:

1. A multivalent antibody binds and recruits a C1 complex.
2. Step 1. ends up initiating a *complement activation cascade*.
3. C5 is cleaved into C5a and C5b as a result of 2.
4. C5a promotes inflammation.
5. C5b recruits the **M**embrane **A**ttachment **C**omplex (i.e., **MAC**)
6. The MAC creates pores in the cell membrane - this leads to cell lysis (and eventually, death).

2.1.2 Recruiting NK Cells and Macrophages

The following process is that of Antibody-Dependent Cellular Cytotoxicity:

Once an antibody is bound to the surface of an antigen, an NK cell's CD16-Fc receptors recognize these antibodies.

As CD-16 are crossed linked, this causes the affected cell to die by apoptosis.

Once IgG antibodies have bound to an antigen, the IgG antibodies bind to the phagocyte via Fc receptors (FcγRI).

These signals (i.e., Fc receptor signals) then causes phagocytosis, thereby killing the antigen.

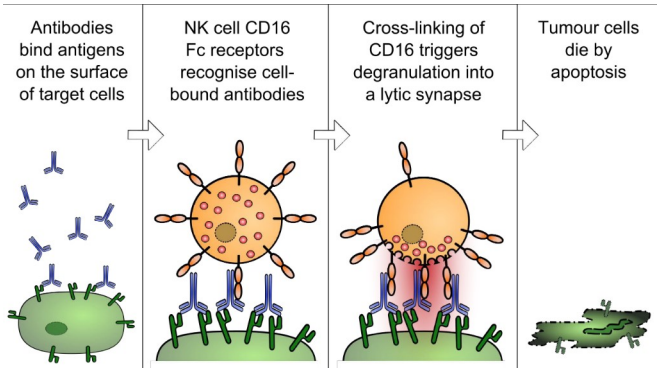

Figure 2.3: How NK Cells are Recruited

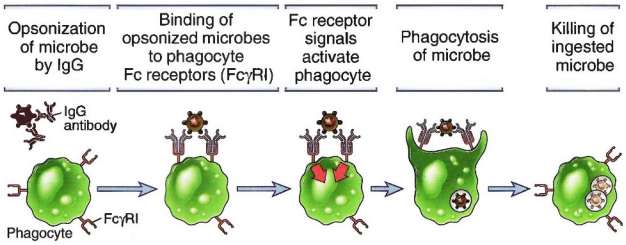

Figure 2.4: Recruitment of Macrophages by IgG Antibodies

2.1.3 Extending Serum Half Lives via Binding of FC Neonatal Receptors (i.e., FcRn)

There are four main processes:

1. Endothelial cells engulf fluids containing IgGs and other plasma proteins from their surrounding environments[3].
2. The early endosomes contain FcRns that bind to the IgG antibodies with a high affinity in spite of the slightly acidic environment (i.e., about pH 6).
3. IgG-FcRn complexes are directed to recycling endosomes while other proteins become degraded in the lysosomes.
4. When IgGs reach cell surfaces, the FcRn binding affinity is reduced due to the pH (i.e., at about 7.4 at this point) - the IgG antibody is released.

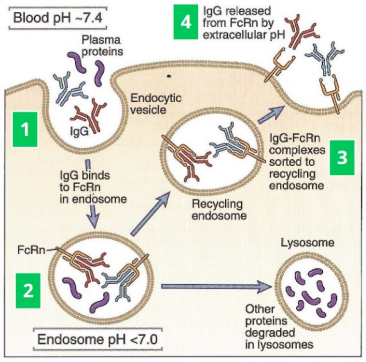

Figure 2.5: How FcRn Binding Works

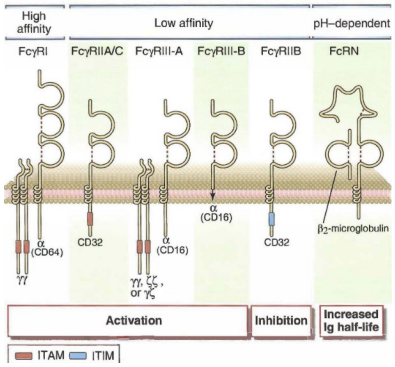

Figure 2.6: Several Fc Receptors

TABLE 13-3 Fc Receptors

FcR	Affinity for Immunoglobulin	Cell Distribution	Function
FcγRI (CD64)	High ($K_d < 10^{-9}$ M); binds IgG1 and IgG3, can bind monomeric IgG	Macrophages, neutrophils; also eosinophils	Phagocytosis; activation of phagocytes
FcγRIIA (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils; eosinophils, platelets	Phagocytosis; cell activation
FcγRIIB (CD32)	Low ($K_d > 10^{-7}$ M)	B lymphocytes, macrophages, dendritic cells, other cells	Feedback inhibition of various cellular responses
FcγRIIC (CD32)	Low ($K_d > 10^{-7}$ M)	Macrophages, neutrophils, NK cells	Phagocytosis, cell activation
FcγRIIIA (CD16)	Low ($K_d > 10^{-6}$ M)	NK cells	Antibody-dependent cell-mediated cytotoxicity
FcγRIIIB (CD16)	Low ($K_d > 10^{-8}$ M); GPI-linked protein	Neutrophils	Phagocytosis (inefficient)
FcεRI	High ($K_d > 10^{-10}$ M); binds monomeric IgE	Mast cells, basophils, eosinophils	Cell activation (degranulation)
FcεRII (CD23)	Low ($K_d > 10^{-7}$ M)	B lymphocytes, eosinophils, Langerhans cells	Unknown
FcαR (CD89)	Low ($K_d > 10^{-6}$ M)	Neutrophils, eosinophils, monocytes	Cell activation?

GPI, glycophosphatidylinositol; *NK*, natural killer.

Figure 2.7: More Details on Fc Receptors

2.2 Fc Receptors

There are several possible receptors available for the Fc fragment of an antibody, of which the following are the most important:

1. **FcγRI**

   This is important for phagocytosis and activation.

2. **FcγRIIIA**

   This is for ADCC.

3. **FcγRIIB**

   This receptor inhibits phagocytosis and cytokine release.

4. **FcRn**

   These extend the serum half-life of the antibody.

2.3 IgG Subclasses

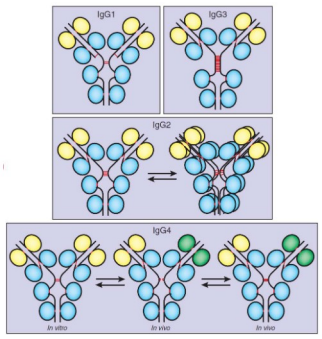

Figure 2.8: Subclasses of the IgG Antibody

There are four main oligomers:

1. **IgG1**

   This is always monomeric bivalent and is the most common subclass for therapeutic antibodies.

[3]This is called **pinocytosis**

2. **IgG2**

   This is in equilibrium between its monomeric bivalent and dimeric tetravalent forms.

   This form is sometimes used to develop therapeutic antibodies.

3. **IgG3**

   This is always monomeric bivalent, but not very commonly used to develop therapeutic antibodies.

4. **IgG4**

   This subclass exists in three different states: monomeric bivalent (left), monomeric monovalent (middle), and mixed monovalent bi-specific forms (right).

   This is used to develop therapeutic antibodies after Fc engineering to stabilize its monomeric bivalent form.

2.4 Steps to Designing a Therapeutic Antibody

There are a total of *five* steps to designing such an antibody:

2.4.1 Step #1: Understanding the Disease

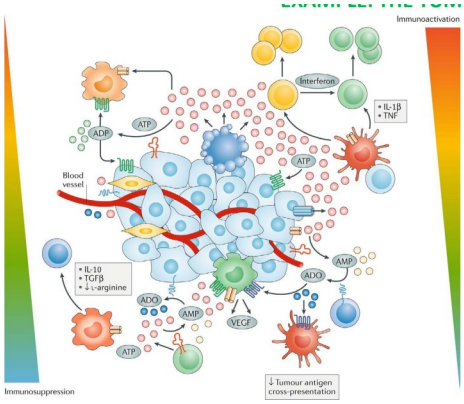

Figure 2.9: An Example Diagram of a Localized Tumor

Antibody therapies are generally based on a solid understanding of the mechanisms that underlie the illness being targeted - in this case, cancer.

The diagram above shows a tumor cell that is surrounded by an excess of ATP molecules (i.e., the pink circles) in vivo. These ATP molecules may promote further release of ATP, cell growth (i.e., uncontrolled mitosis), and also immune and inflammatory cell responses.

2.4.2 Step #2: Identifying the Target Molecule and the Mode of Action

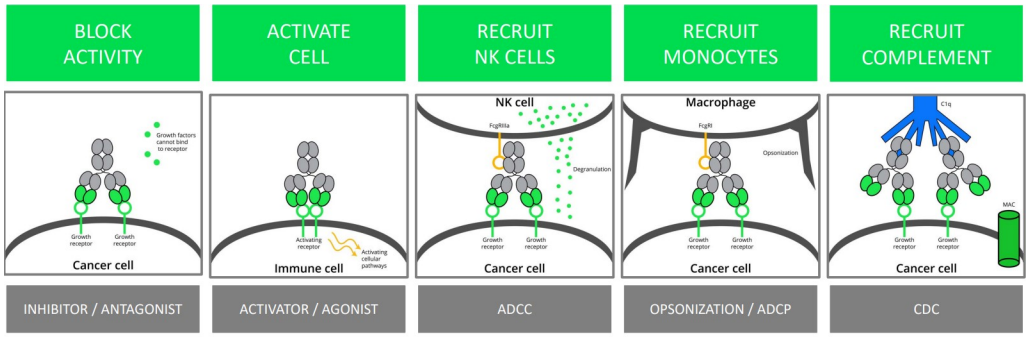

Figure 2.10: Possible Modes of Action of an Antibody

Depending on the intended modes of action and / or the target regions of the target (i.e., the epitope) for the antibody in question, a different subclass of IgG antibodies might need to be used.

Recall that IgG1 antibodies excel in targeting carbohydrates while IgG2 antibodies excel in targeting polysaccharides (e.g., sugar coatings).

2.4.3 Step #3: Generating the Antigen Binding Fragments

BS4019 lists two common methods of performing this step:

2.4.3.1 Using Animals

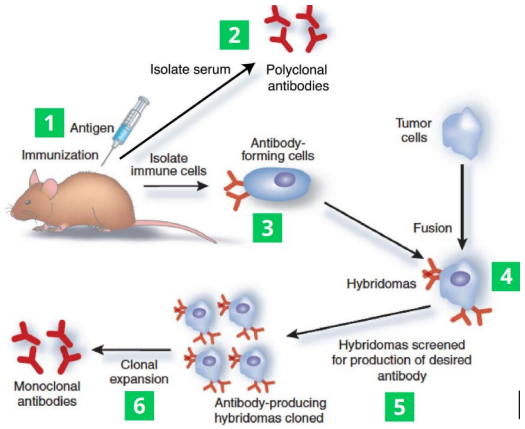

Figure 2.11: Using Mice as Model Organisms for Antibody Generation

In this procedure, an antigen is first injected into an animal (i.e., step 1). A serum (i.e., step 2) can then be extracted to yield a **polyclonal** antibody solution[4].

Once antibody-producing B-cells can be extracted from the animal, these same B-cells are also fused with tumor cells to form **hybridoma cells** (i.e., step 4). These cells divide indefinitely and in doing so, produce antibodies.

These antibodies are then screened[5] to filter out the correct antibody for mass production before they are *clonally expanded* to form monoclonal antibodies.

2.4.3.1.1 An Alternative to Step 4

Bioinformatics may also be used to generate a suitable sequence to be fused with tumor cells' genomes.

The VH and VL regions of the Fab fragment of the target antibody can then be amplified via in vivo methods (e.g., phage displays).

2.4.3.2 Phage Libraries

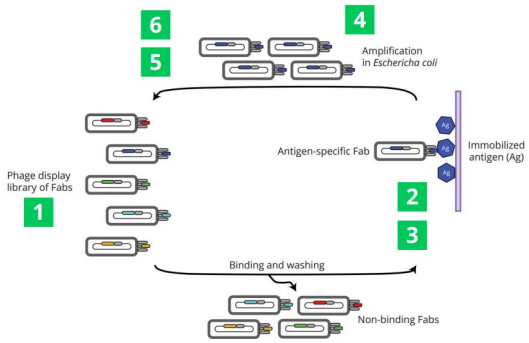

Figure 2.12: Antibody Production Using Bacteriophages

A library that contains about 10^{10} is first displayed at the surface of a bacteriophage before it is exposed to an antigen (which is typically the protein of interest that has been immobilized on a surface). In doing so, non-binding phages are washed away.

The binding phages then go on to infect *E. coli* cells - this process happens about two to six times. An ELISA assay is typically used after this to ensure binding. The phages may also be sequenced to identify Fab sequence.

[4]While this can be used in practice, the resulting serum will be very crude and may not be as effective or safe as using a solution of monoclonal antibodies.

[5]This is because many different kinds of antibodies can be produced from the hybridoma cells.

2.4.3.2.1 Alternative Steps

Instead of developing a phage library, a yeast or mRNA display can be used instead.

Otherwise, next-generation sequencing can also be used in step 4.

2.4.3.3 Step #4: Selecting an Appropriate IgG Subclass

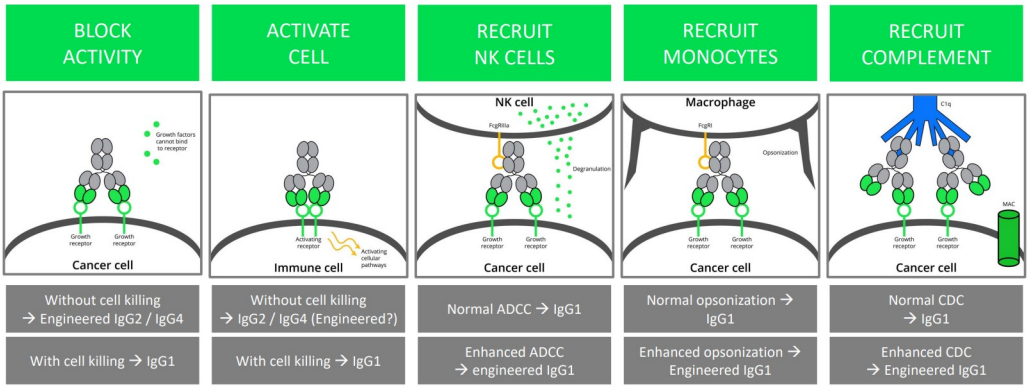

Figure 2.13: Potential Uses and Side Effects of Different IgG Antibody Subclasses

Recall that IgG3 antibodies are generally never used in therapeutic antibody production due to its unfavorable diamond configuration.

However, both IgG1 and IgG2 / IgG4 antibodies can be used with the following side effects for:

1. **Blocking Activity**

   IgG2 / IgG4 antibodies can be used to accomplish this without destroying the cell.

   IgG1 antibodies can be used too, but they cause cell death.

2. **Cell Activation**

   IgG2 / (engineered) IgG4 antibodies can be used to do this without killing the cell.

   IgG1 antibodies can be used for this purpose too, albeit they kill the cell.

3. **Recruiting NK Cells**

   IgG1 antibodies can be used for this.

   Engineered IgG1 antibodies can lead to enhanced ADCCs.

4. **Recruiting Monocytes**

   IgG1 antibodies are used for this.

   Engineered IgG1 antibodies can lead to enhanced opsonisation[6].

5. **Recruiting Complements**

   Normal CDCs can help recruit IgG1 antibodies.

   Likewise, enhancing CDCs can help recruit enhanced IgG1 antibodies.

2.4.4 Step #5: Humanizing Antibodies

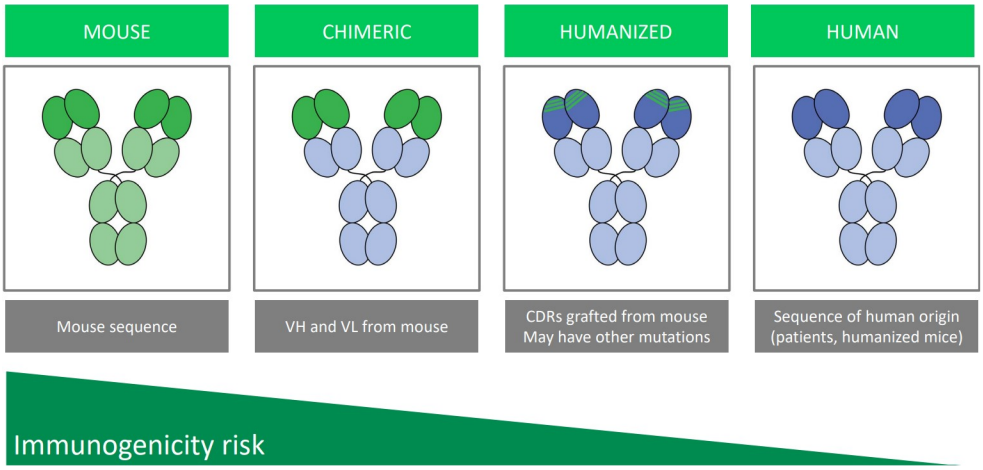

Figure 2.14: Immunogenicity Risk of Antibody by Humanization Status

Humanizing antibodies decreases the risk of **immunogenicity**: the event that the body's immune system attacks a non-self molecule.

Chimeric antibodies are antibodies whose VH and VL regions from mice antibodies have been grafted onto a human antibody's Fab fragment.

In a **humanized antibody**, parts of the Fab's CDR have been grafted on from mouse antibodies.

Some examples of FDA-approved antibodies are shown in the above table. Depending on the suffix of the antibody's name, one can guess the origin of the antibody - most notably:

1. "-lumab" refers to a human antibody.
2. "-umab" refers to a mouse antibody.
3. "-ximab" refers to a chimeric antibody.

[6]This is a process whereby a pathogen is surrounded by antibodies.

Table 1 FDA approved tumor-therapeutic antibodies [2–24]

Targeted drugs	IgG subclass	Target	Indications	Mechanism of action
Rituximab (Rituxan)	IgG1	CD20	NHL	ADCC/CDC
Trastuzumab (Herceptin)	IgG1	Her2	Breast cancer	Growth signal blocking; ADCC
Cetuximab (Erbitux)	IgG1	EGFR	mCRC	Growth signal blocking; ADCC
Bevacizumab	IgG1	VEGF	Solid tumors	Angiogenesis inhibition
Panitumumab (Vectibix)	IgG2	EGFR	mCRC	Growth signal blocking
Ofatumumab (Arzerra)	IgG1	CD20	CLL	ADCC/CDC
Alemtuzumab (Campath)	IgG1	CD52	CLL	ADCC/CDC
Denosumab (Xgeva)	IgG1	RANKL	Bone tumor	Growth signal blocking
Ipilimumab (Yervoy)	IgG1	CTLA-4	Solid tumors	Depleting Treg cells
Pertuzumab (Perjeta)	IgG1	Her2	Breast cancer	Growth signal blocking
Obinutuzumab (Gazyva)	IgG1	CD20	CLL	ADCC; apoptosis induction
Ramucirumab (Cyramza)	IgG1	VEGFR2	Solid tumors	Angiogenesis inhibition
Pembrolizumab (Keytruda)	IgG4	PD-1	Solid tumors	Neutralizing inhibitory signal in T cells
Nivolumab (Opdivo)	IgG4	PD-1	Solid tumors	Neutralizing inhibitory signal in T cells
Dinutuximab (Unituxin)	IgG1	GD2	Neuroblastoma	ADCC/CDC
Daratumumab (Darzalex)	IgG1	CD38	Multiple myeloma	ADCC/CDC; apoptosis induction
Elotuzumab (Empliciti)	IgG1	SLAMF7	Multiple myeloma	ADCC,direct activation of NK cells
Atezolizumab (Tecentriq)	IgG1	PD-L1	Solid tumors	Neutralizing inhibitory signal in T cells
Avelumab (Bavencio)	IgG1	PD-L1	Solid tumors	Neutralizing inhibitory signal in T cells; ADCC
Durvalumab (Imfinzi)	IgG1	PD-L1	Solid tumors	Neutralizing inhibitory signal in T cells
Mogamulizumab (Poteligeo)	IgG1	CCR4	CTCL	ADCC
Cemiplimab (Libtayo)	IgG4	PD-1	Solid tumors	Neutralizing inhibitory signal in T cells

Figure 2.15: Some Examples of Therapeutic Antibodies

2.4.5 Step #6: Validating Molecules

See the following chapter for more information!

2.5 Phages and Library Displays

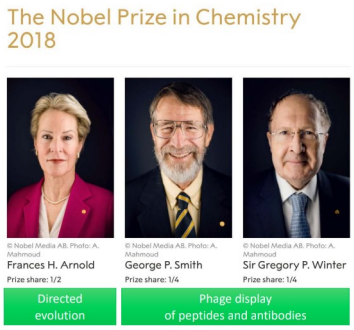

Figure 2.16: Some Nobel Prize Winners for the Development of Phage Libraries

A possible alternative to using animals to generate antibodies include using phage libraries (as outlined in section 2.4).

The individuals behind the development of phage libraries have been awarded Nobel prizes (see above image).

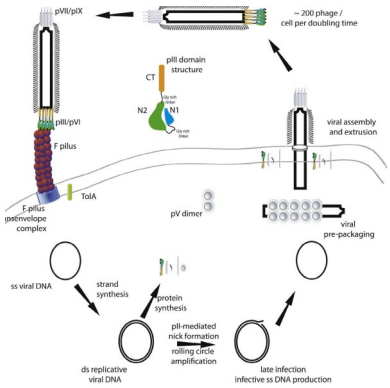

Figure 2.17: Life Cycle of a Bacteriophage

2.5.1 Life Cycle of a Phage

It is believed that M13 phage coat proteins are formed in the periplasm before they are packed into the phage in question.

Note that other phages (e.g., bacteriophage T7) can also be packed into the cytoplasm.

2.5.2 Phage Display Formats

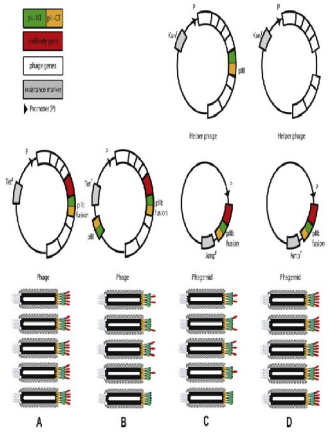

Figure 2.18: Possible cDNA Outcomes from Recombination

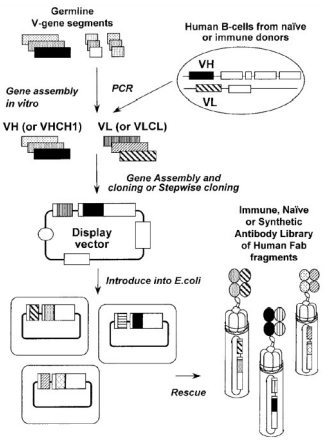

Figure 2.19: Creating a Gene Library from Bacteriophages' cDNA

2.5.3 Library Creation from cDNA

2.5.4 Kunkel Mutagenesis

This was also prof. Asial's focus during his PhD.

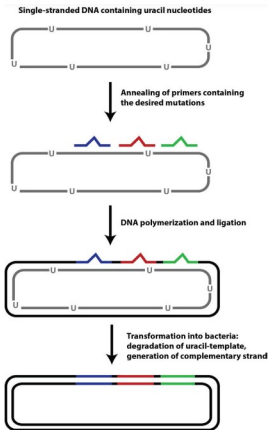

Figure 2.20: Kunkel Mutagenesis Outlined