

BS4019: Discovery of Therapeutic ANtibodies

Kevin Fo

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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

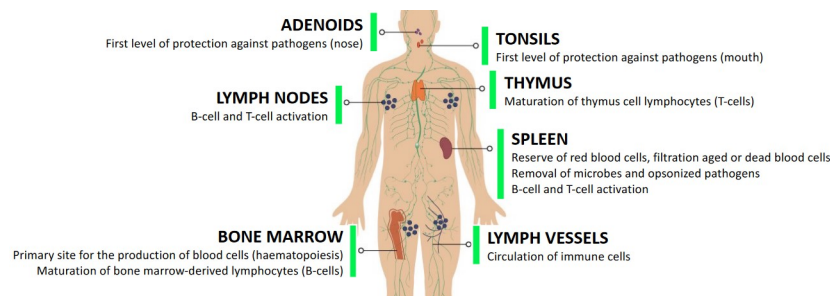


Figure 1.1: Various Organs of the Human Immune System

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).

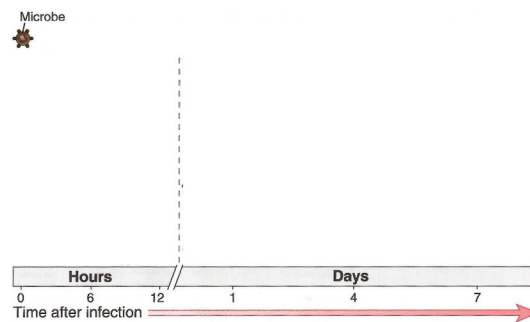


Figure 1.2: Timeline of Infection

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.

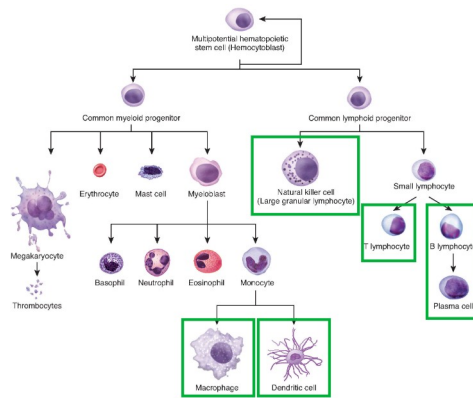


Figure 1.3: Cells of the Immune System

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.

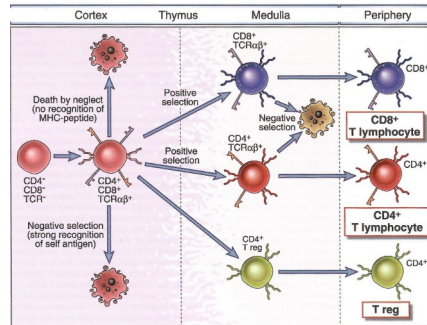


Figure 1.4: Possible T-Cells from Differentiation

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

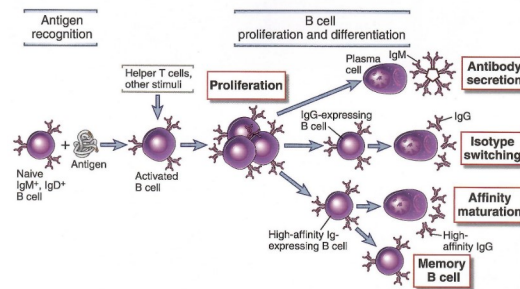


Figure 1.5: B-Cell Differentiation in the Human Immune System

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

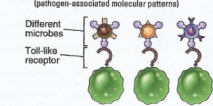
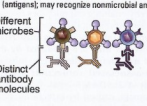
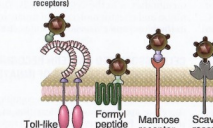
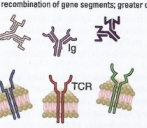
	Innate Immunity	Adaptive Immunity
Specificity	For structures shared by classes of microbes (pathogen-associated molecular patterns)	For structural detail of microbial molecules (antigens); may recognize zoonicrobial antigens
	 <p>Different microbes Toll-like receptor</p>	 <p>Different microbes Distinct antibody molecules</p>
Receptors	Encoded in germline; limited diversity (pattern recognition receptors)	Encoded by genes produced by somatic recombination of gene segments; greater diversity
	 <p>Toll-like receptor Formyl peptide receptor Mannose receptor Scavenger receptor</p>	 <p>Ig TCR</p>
Distribution of receptors	Nonclonal: identical receptors on all cells of the same lineage	Clonal: clones of lymphocytes with distinct specificities express different receptors
Discrimination of self and non-self	Yes; healthy host cells are not recognized or they may express molecules that prevent innate immune reactions	Yes; based on elimination or inactivation of self-reactive lymphocytes; may be imperfect (giving rise to autoimmunity)

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-cell receptors** (i.e, **TCRs**) 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	T cell receptor (TCR)*	MHC molecules*
	Immunoglobulin (Ig)		
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 $\alpha 1$ and $\alpha 2$ domains (class I MHC) and $\alpha 1$ and $\beta 1$ 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	$K_d 10^{-6} - 10^{-11}$ M; average affinity of Ig increases during immune response	$K_d 10^{-6} - 10^{-7}$ 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

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

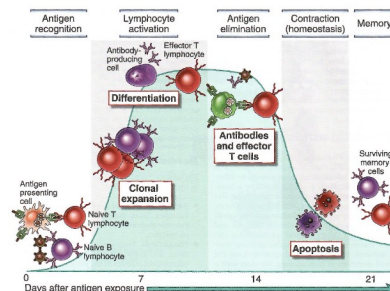


Figure 1.8: Activation of the Adaptive Immune System

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

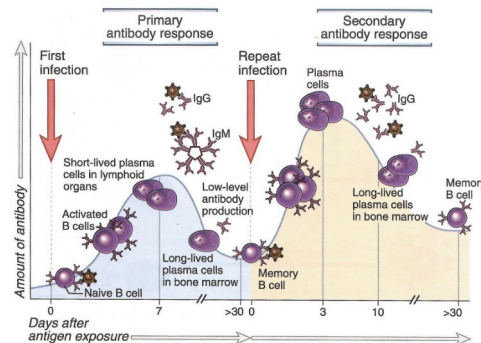


Figure 1.9: Amount of Antibodies over Time

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.

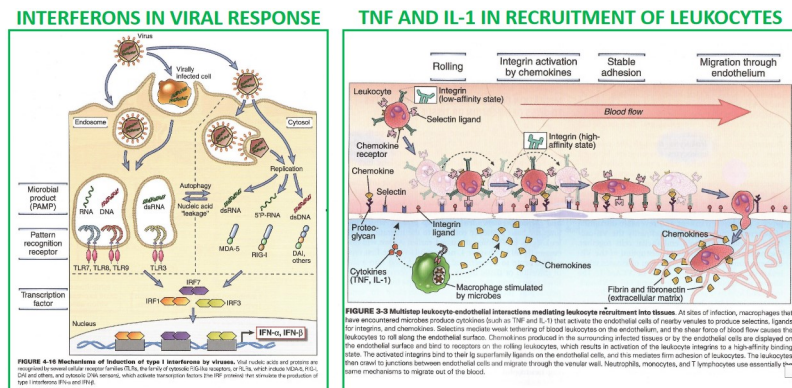


Figure 1.10: Examples of Cytokines in Various Scenarios

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

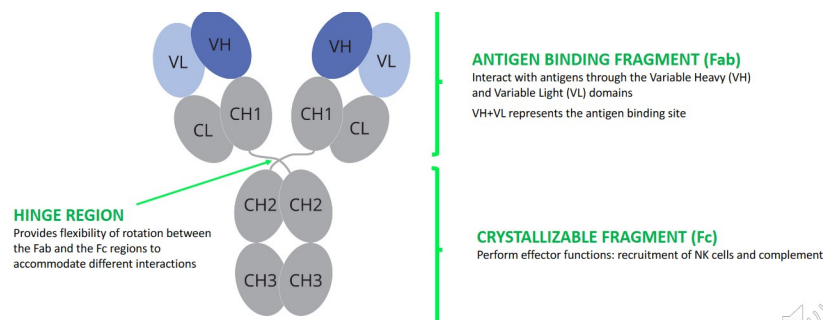


Figure 1.11: Basic Structure of an Antibody

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

The **antigen binding fragments (Fab)** interact with antigens via **variable heavy** (i.e., **VH**) and **variable light** (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

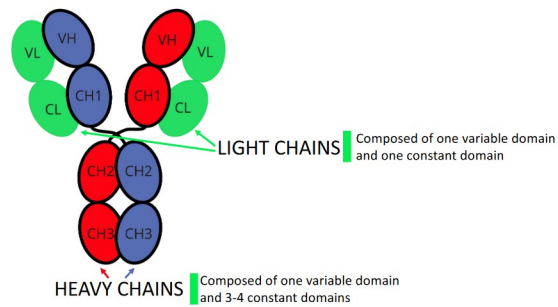


Figure 1.12: Light and Heavy Chains of an Antibody

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

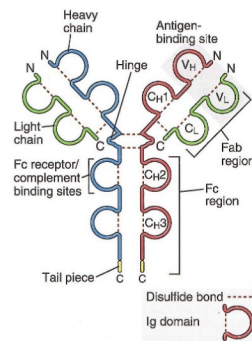


Figure 1.13: Bonds in an Antibody

Antibodies are stabilized 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

Isotype of Antibody	Subtypes (if Clinically)	Serum Concentration (mg/dL)	Serum Half-life (days)	Secreted Form	Functions
IgA	IgA1, IgA2 (α1 or α2)	2.5	6	Monomer; dimer; also monomer, dimer	Mucosal immunity
IgD	None (δ)	Trace	3	Monomer	Naive B cell antigen receptor
IgE	None (ε)	0.05	2	Monomer	Defense against helminths; parasites, immediate hypersensitivity
IgG	IgG1, IgG2, IgG3, IgG4 (γ1, γ2, γ3, γ4)	12.5	23	Monomer	Opsonization, complement activation, antibody-dependent cell-mediated cytotoxicity, neutralization, feedback inhibition of B cells
IgM	None (μ)	1.5	5	Pentamer	Naive B cell antigen receptor (monomer level), 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., CDRs)

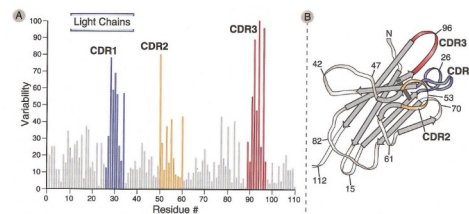


Figure 1.16: CDRs in an Antibody

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.