BS4019: Antibodies

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Preface

About Dr. Ignacio Asial

Dr. Ignacio is an Argentinian who finished his High School, his Bachelors', and his Masters' in France before coming to Singapore for his PhD (at NTU).

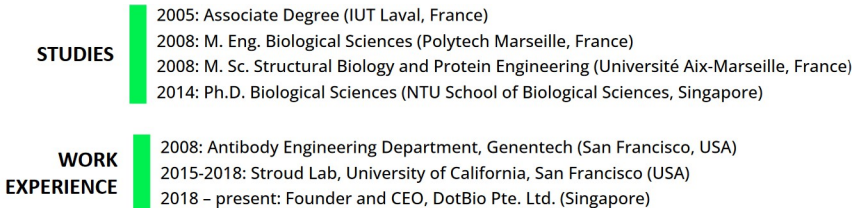

Figure 1: Dr. Ignacio Asial's Background and Credentials

Dr. Ignacio has since gone on to found his own company DotBio

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

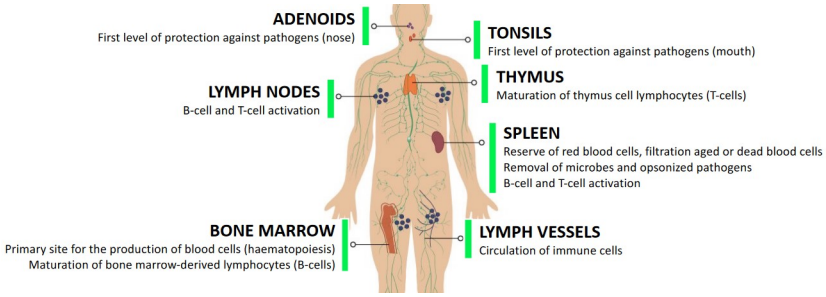

Figure 1.1: Various Antibodies in the Human Immune System

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).

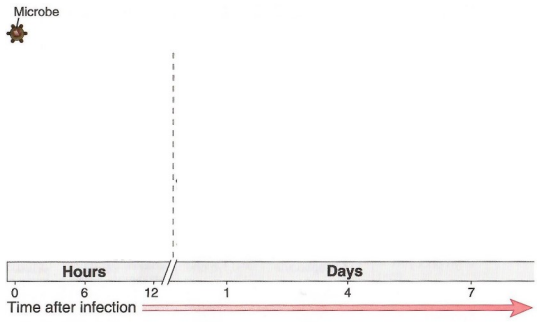

Figure 1.2: Timeline of Infection

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.

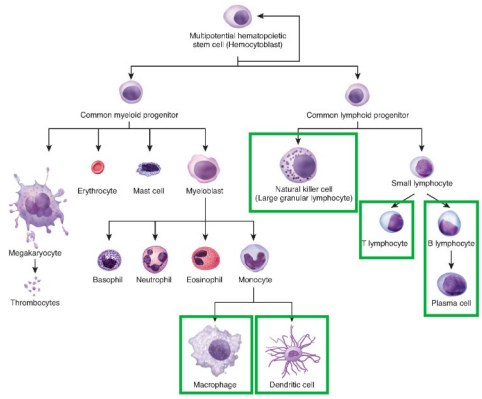

Figure 1.3: Cells of the Immune System

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.

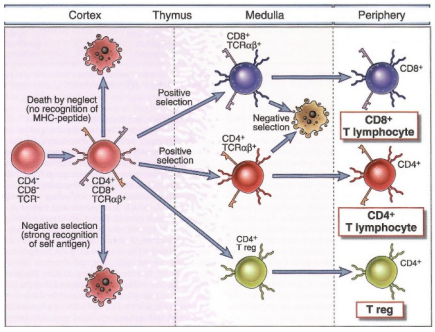

Figure 1.4: Possible T-Cells from Differentiation

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

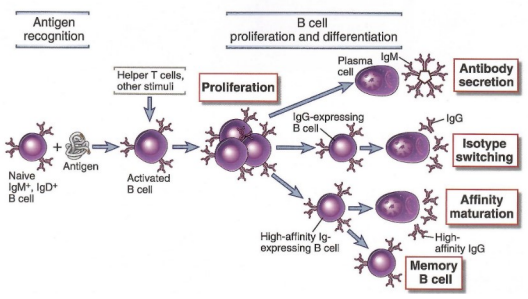

Figure 1.5: B-Cell Differentiation in the Human Immune System

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

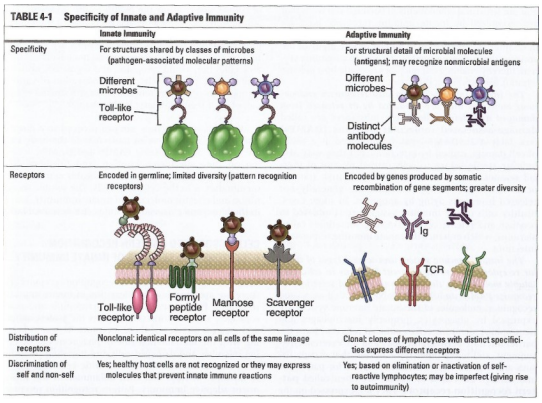

Figure 1.6: Structures Involved in Innate and Adaptive Immunity

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

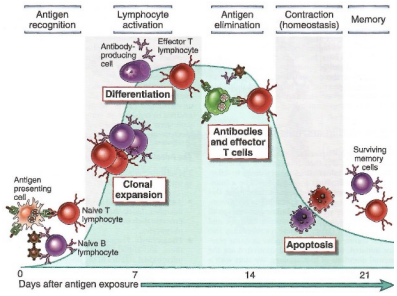

Figure 1.8: Activation of the Adaptive Immune System

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

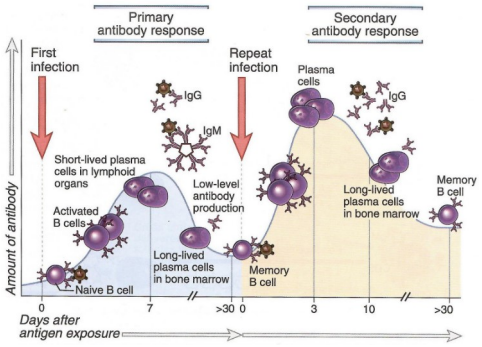

Figure 1.9: Amount of Antibodies over Time

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.

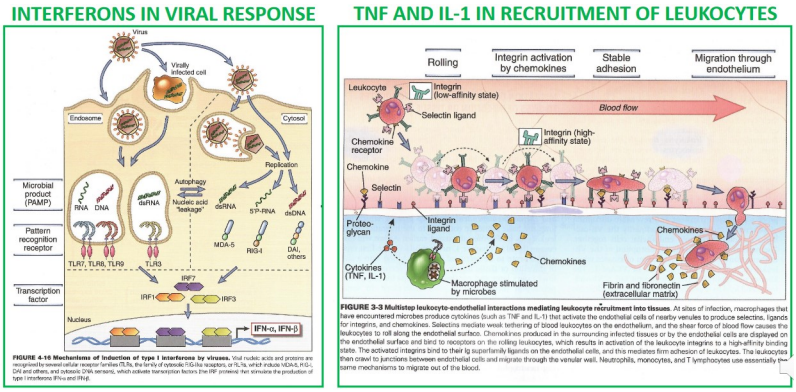

Figure 1.10: Examples of Cytokines in Various Scenarios

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

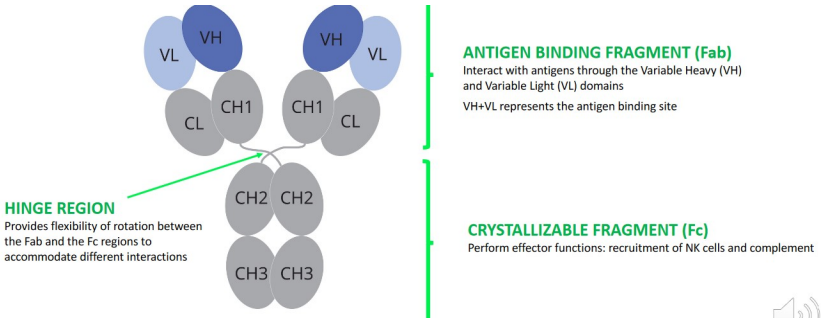

Figure 1.11: Basic Structure of an Antibody

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

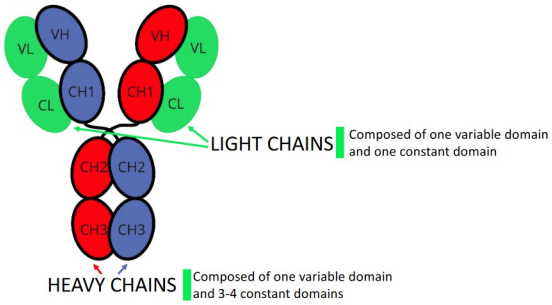

Figure 1.12: Light and Heavy Chains of an Antibody

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

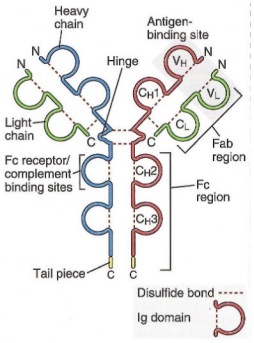

Figure 1.13: Bonds in an Antibody

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

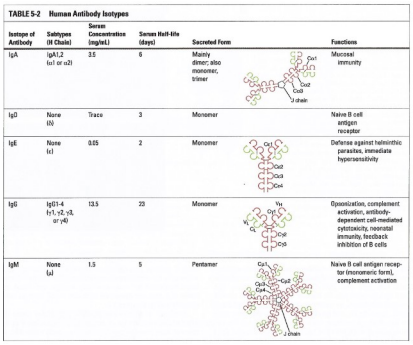

TABLE 5-2 Human Antibody Isotypes

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

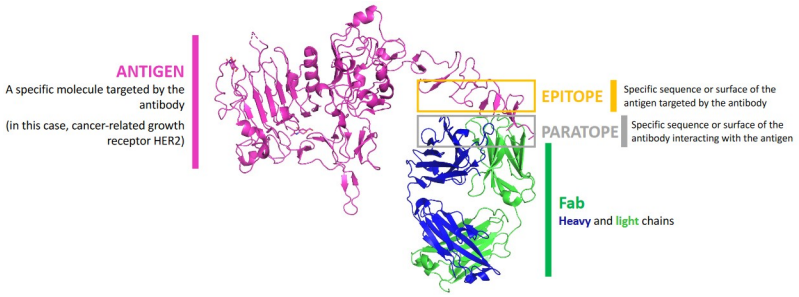

Figure 1.15: Structure of Antibody Labelled

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)

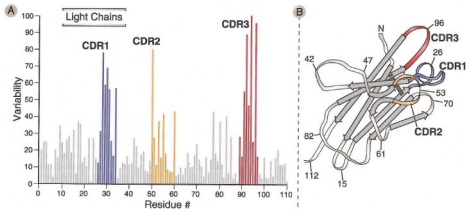

Figure 1.16: CRDs in an Antibody

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.