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Antibodies
(Recommended reading: Abbas et al., 6th edition, Chapter 3; Chapter 4;
Janeway et al., 5th edition, Chapter 3)

There was a king reigned in the East:
There, when kings will sit to feast,
They get their fill before they think
With poisoned meat and poisoned drink.
He gathered all that springs to birth
From the many venomed earth;
First a little, thence to more,
He sampled all her killing store;
And easy, smiling, seasoned, sound,
Sat the king when healths went round.
They put arsenic in his meat
And stared aghast to see him eat;
They poured strychnine in his cup
And shook to see him drink it up:
They shook, they stared as white's their shirt.
Them it was their poison hurt.
-I tell the tale that I heard told.
Mithridates he died old.

From "Terence, this is stupid stuff"
By A.E. Housman

Antibodies protect us from a vast variety of pathogens. Indeed the antibody repertoire is immense - the binding or *combining sites* of antibodies may be able to recognize somewhere in the range of 10 million different shapes. Antibodies share with agglutinins all the features that contribute to the elimination of pathogens, and can also contribute to host defense in some ways that the innate immune system cannot.

Antibodies are also called **immunoglobulins**. A very crude electrophoretic fractionation (separation on the basis of charge) of serum proteins separates albumin from other serum proteins collectively called globulins. The globulins are also further characterized on the basis of charge as α, β and γ -globulins, γ-globulins being the most positively charged. This latter fraction was discovered to be made up largely of antibody molecules. The existence of antibody secreting tumors known an myelomas or plasmacytomas greatly facilitated the study of immunoglobulins and their structure. Tumors are in general derived from a single cell. A single cell and its progeny are generally referred to as a **clone** and tumors are therefore **clonal** (or

monoclonal) outgrowths. Myelomas and plasmacytomas are derived from differentiated antibody secreting B lymphocytes known as plasma cells These plasma cell tumors each produce large amounts of a single or **monoclonal** antibody.

The portions of the heavy chain and light chain that are involved in antigen recognition are at the N-terminal ends and are referred to as **V (or variable) domains.** The remaining portions of the heavy and light chains are referred to as **constant (or C) regions**. Each light chain constant region contains a single **constant domain** (CL). A **domain** refers to a portion of a protein which can be separated from the rest of the molecule and still fold into its correct shape. The domains in immunoglobulin molecules have a typical three dimensional structure which is now referred to as an immunoglobulin domain and is found in a variety of proteins (many of which existed before the evolution of immunoglobulins). Each heavy chain constant region is made up of three or four domains (CH).   The portion of each heavy chain that is associated with the light chain is the VH domain and the CH1 domain. A cysteine residue in the CH1 domain forms a covalent disulfide bridge with a cysteine residue towards the C-terminal end of the CL domain. When one class of immunoglobulin molecules known as IgG molecules are cleaved with a proteolytic enzyme called papain, these antibodies are cleaved into two identical fragments known as Fab (**F**raction **a**ntigen **b**inding) fragments and a single Fc (**F**raction **c**rystallizable) fragment. Cleavage with pepsin yields an F(ab)2 fragment and an Fc fragment. Each Fab fragment corresponds to an arm of the antibody Y and is made up of a light chain covalently associated (via a disulfide bridge) with a portion of the heavy chain containing the VH and CH1 domains. The Fc portion corresponds to the stem of the Y and is a dimer of the CH2 and CH3 domains of IgG covalently united via disulfide bridges between the two heavy chains.

The variable domains of both heavy and light chains contain a large number of residues that are identical or highly conserved between different antibodies. These are referred to as **framework regions.** The variable nature of these domains is contributed to by stretches of amino acids that differ from one antibody variable domain to another. These residues make up three **hypervariable regions** or **complementarity determining regions** or **CDR**s. In both the heavy chain and the light chain CDR3 is the most variable of the hypervariable regions. Every constant or variable region domain forms an immunoglobulin fold which looks a lot like two very similar slabs slapped together at a slight angle. Each slab is made up of strands of a polypeptide strung up and down in succession to make a structure known as a **β-sheet.** The immunoglobulin fold itself is made up of these two slabs of β-sheets compressed against each other (known as an antiparallel β-barrel). At the top and bottom of this sandwich are loops that maintain continuity between individual strands of these β-sheets. When one examines the structure of a variable domain the three CDRs form loops of highly variable configuration that protrude from the "top" of a very conserved immunoglobulin fold structure. It is these very variable CDR loops of a VH and a VL domain that combine to form a binding site for antigen.

If an antibody is directed against a small antigenic determinant or epitope the binding site formed by the three CDR loops may resemble a relatively tight pocket into which the epitope might fit. If the epitope is somewhat larger such as the rough terrain forming an antigenic "patch" on the surface of a protein, the combining site of the antibody may be formed by an outward splaying of the six CDR loops. The six hypervariable regions from the heavy and light

chains combine to form a complementary surface to that of the protein antigenic determinant with some of the little hills on one surface dipping into the valleys of the other and vice versa

Antibodies exist as a number of classes or isotypes (defined on the basis of their heavy chains). The antibody isotype that is made early in an immune response is of the IgM class. Later in an immune response a given B lymphocyte may "switch" from producing IgM antibodies against a specific antigen, to producing antibodies of other isotypes, such as IgG, IgA or IgE, also directed against the original antigen. *Class switching* of antibodies typically occurs in responses to protein antigens that are driven in part by signals from T lymphocytes. Another important phenomenon that tends to occur in immune responses driven by T cell derived signals is a process by which mutations are introduced into the genes encoding the variable portion of the antibody heavy and light chains. This process is referred to as *somatic mutation*. As we will discuss in later lectures, the selection by antigen of B lymphocytes whose mutated receptors "fit" the antigen most tightly results in the further differentiation of only those B cells which make antibodies of high affinity toward their cognate epitopes. This process is also described as *affinity maturation*. Since class switching occurs in cells that also undergo affinity maturation, IgG, IgE and IgA antibodies are likely to have combining sites which fit the antigenic determinant more tightly than the original IgM antibody made by the same B cell before it switched and mutated its immunoglobulin genes. These issues and the mechanism involved in class switching and somatic mutation will be discussed in the lecture on B cell activation

Although IgM antibodies tend to have individual binding sites that have a lower *affinity* for antigen than the corresponding combining sites on IgG antibodies, the valency of secreted IgM antibodies permits them to bind to antigens quite well. IgG antibodies are each made up of two identical heavy chain molecules and two identical light chain molecules. When purified by being sedimented at very high speeds in an ultracentrifuge, *IgG* molecules are described as being *7S antibodies*. S represents a Svedberg unit, named after the Swedish scientist who pioneered the use of the ultracentrifuge and is a measure of the hydrodynamic properties of a protein which generally reflect the size of the protein being examined. Secreted *IgM* antibodies are generally referredto as *19S immunoglobulins*. They are made up of pentamers and hexamers of the basic 7S unit. Pentameric IgM therefore contains ten combining sites and is able to hold on to an antigen more tightly because of this multiplicity of sites, even though individual sites are of relatively low affinity. It is this overall ability to bind antigen which is measured as *avidity*.

The structural features of each antibody class is determined by a distinct type of heavy chain protein. The V_H domain of a given antibody is originally physically in continuity with C_H domains of the μ heavy chain. Antibodies of each of the other classes and sub-classes have distinct heavy chain proteins. Although a given B cell starts out making IgM and may later secrete IgG, IgA or IgE it always makes either a κ or a λ light chain. These two types of light chain are referred to as light chain classes or isotypes. There are no major functional differences that can be attributed to κ or λ light chains. Immunoglobulin heavy chains which lack C-terminal hydrophobic anchors are components of **secretory immunoglobulins** which we generally call antibodies. The membrane bound form of the heavy chain combines with light chains to form **membrane immunoglobulins** which participate in the recognition of antigen by B cells. Very small molecules (like drugs or monosaccharides) can be recognized by antibodies

but cannot trigger B cells. They are called **haptens**. Haptens are "antigens" but are not "**immunogens**". A multivalent form of a hapten may be able to crosslink the B cell receptor and activate a B cell to divide and to differentiate into a plasma cell that secretes antibodies. Multivalent antigens are known as **T-independent antigens**.

Ig tetramers in dimeric IgA and pentameric IgM are complexed together covalently associated to a J chain. The J chain facilitates the transport of secretory IgA and IgM acroos epithelial bariiers by a process called transcytosis. The polyIg receptor on epithelial cells binds to the J chain containing multimer on the basolateral side, and transports the immunoglobulin to the luminal side of the epithelium. The extracelluar domain of the poly Ig receptor is proteolytic ally cleaved and it stays bound to dimerism IgA in the lumen and is known as the "secretory component".

Membrane immunoglobulins also function as endocytic receptors that recognize B cell epitopes and facilitate the internalization of antigens. Protein antigens that are specifically internalized may be cleaved in a lysosome-like compartment into peptides that are presented to T cells by MHC class II molecules. Protein antigens that carry a B cell epitope (the haptenic determinant) physically linked to a T cell epitope (the **carrier** determinant) represent **T-dependent antigens**.

Summary:

- Antibodies or immunoglobulins (Igs) are present in the plasma with an enormous degree of diversity (roughly 10^7 different shapes) that can recognize diverse antigenic structures.

- Much of our understanding of antibody structure comes from myeloma proteins. Myelomas are plasma cell tumors that can secrete large quantities of monoclonal antibodies. (Plasma cells are "end" cells of the B lineage).

- Antibodies are at least bivalent. IgG molecules consist of two heavy chains (HC) and two light chains (LC) in a Y-shaped form. These chains are held together by disulfide bridges.

- Each HC and LC contains variable domains that recognize a specific antigen and constant regions that perform effector functions (e.g. recognition for phagocytosis or for binding to complement).

- The variable domains contain both framework regions (that are highly conserved) as well as three hypervariable regions ("complementarity determining regions") on each chain. CDR3 is the most variable.

- IgA and IgM exist as multimers (dimers for IgA and pentamers for IgM) which are linked by disulfide bridges to a J chain.
- IgG isotypes and IgE are monomeric
- IgM tends to bind with lower affinity to antigen than other isotypes, however its pentameric structure increases its overall ability to bind antigen (avidity).
- Immunoglobulins are generated either as secretory proteins (antibodies) or membrane bound proteins which function as antigen receptors
- Membrane immunoglobulins may recognize multivalent antigens (T-independent antigens) when they function as signaling receptors
- Small monovalent antigen which can be recognized by antibodies but which cannot initiate an immune response are called haptens
- Membrane immunoglobulins may also function as internalization receptors which internalize protein antigens (T-dependent antigens) which are processed and presented to T cells
- T-dependent antigens are made up of a B cell epitope (the haptenic determinant) which is physically linked to a T cell epitope (the carrier determinant).

Objectives/ Study questions:

1. Review some basic concepts in protein structure. What do you mean by primary, secondary, tertiary, and quaternary structure?

2. Outline the domain structure of an IgG antibody.

3. What is the difference between the structure of IgG, IgM, and IgA?

4. How are membrane and secretory antibodies generated?

5. Define idiotypes, allotypes, and isotypes.

6. What are the functions mediated by the effector domains of antibodies?

7. Define hapten, antigen, immunogen, T-independent antigen, and carrier determinant.