Lecture 3: Immunoglobulin Structure and Function
Chapter 4: Immunology, a short course.

- **Antigens:** Foreign material that is recognized by the immune system. Type of response (B-cell versus T-cell) depends on the antigen.
- **Immunogen**: Foreign material that produces an immune response (B- and T-cell activation)
- **Hapten**: Foreign material that is reccognized by the immune system, but can only function as an immunogen when complexed to other immunogens.
- **Antibodies**, or immunoglobulins:
  - Activated B cells produce soluble antibodies.
  - Unactivated and memory B-cells produce surface, membrane-bound, antibodies.
  - The membrane form is associated with two copies of Ig_α and Ig_β, giving the B-cell receptor.
  - Antibodies can recognize an extremely diverse set of antigens.
  - A *single* B-cell produces antibodies that are *homogenous* in their specificity. This is one example of **Allelic exclusion** in the immune system. Although two copies of the gene are present (maternal and paternal), only one is used to express the protein.

Historical and Biochemical Evidence for Immunoglobulin structure.

1. Electrophoretic separation of serum proteins yields albumin, α, β, γ globulin, in that order. γ globulin levels were increased in immunized animals and could be decreased by incubation with specific antigens.
2. papain (protease) cut γ-globulin into two identical Fab fragments (fragment-antigen binding) and Fc (fragment that crystallized).
3. pepsin (protease) cut γ-globulin into a single 100 KDa fragment $F(ab')_2$, consisting of two Fab domains.
4. Reduction of disulfide bonds showed the presence of four chains, two light (25 KDa) and two heavy chains (50 KDa).
5. Anti-Fab antibodies bound to both heavy and light chains
6. Anti-Fc antibodies bound only to heavy chains.

These data lead to proposal of a Y-shaped structure by Porter in **1962**, many years before the 1st crystal structure was known.

Structure and Function of IgG: Prototypical Antibody Structure:

Quaternary Structure:

- 2 Light Chains: V_L domain (110 residues), Constant Domain, C_L (110 residues).
  Two forms of light chains, λ (lambda) and κ (kappa).
- 2 Heavy Chains: IgG V_H domain has C_H1, C_H2, C_H3.
  Five different forms: γ, α, μ, δ, ε.
- Class of an immunoglobulin is defined by its type of heavy chain: IgG(γ), IgA(α), IgM(μ), IgD(δ), IgE(ε). These are termed **Isotypes**.
- V domains pair in heavy and light Chains, as do C_L and C_H1.
- Variable region recognizes antigen
- Constant region has effector functions.

- C_L and C_H1 linked by disulfide bond
- C_H2 in each heavy chain linked by disulfide bond.
- Carbohydrate linked to C_H2 domain.
- Region between CH1 and CH2 is non-globular and composed of Cys and Pro, this **hinge region** is thought to provide conformational flexibility for the two Fab domains.
- **Membrane bound** forms contain a transmembrane segment followed by a very short cytosolic segment. This is generated by alternative splicing/polyadenylation of the mRNA.

Tertiary Structure:

- Each domain (e.g. V_L, C_H3) consists of 7 stranded (4+3) β-sandwich, crosslinked by a disulfide bond. This structure is termed the **Immunoglobulin fold** and is found in many proteins that participate in the immune response.

Primary Structure:

- Constant regions have the same sequence within a class of antibody. Haplotype differences can occur , but the human population is not very polymorphic.
- Variable regions differ from antibody-to-antibody, generating *diversity*.
- Extensive sequence variability found in three segments of both V_L and V_H. These are *called hyper-variable regions*. Since these regions are also primarily involved with binding antigen they are also referred to a *complementary-determining-regions (CDR)*.

Antibody-Antigen Interactions:

- Each pair of associated V_L and V_H domains can bind antigen. Therefore IgG can bind 2 antigens.
- Usually all 6 (3L and 3H) CDRs are used.

Diversity: Antibody diversity is generated by:

- Multiple genes encoding both VL and VH
- Segmental joining of additional DNA segments to form the mature V_L and V_H genes
- Addition of nucleotide bases during the joining event
- Random association of Heavy and light chains (facilitated by the V_C, C_H1 disulfide bond)
- Somatic (body) mutation of mature heavy and light chain genes after antigen stimulation generate higher affinity antibodies.

Antibody-Hapten Interactions: (e.g. dinitrophenyl, phosphocholine, cocaine, PCP, human chorionic gonadotrophin -HCG)

- Antibodies generated by attaching hapten to a carrier protein to make it immunogenic
- Resultant antibodies can recognize hapten with useful specificity and affinity.
- Interaction between antibody and immunoglobulin generally involves a deep binding pocked, utilizing 4-6 of the CDRs.
- Applications of Antibody-Hapten Interactions:
  1. Antibodies against HCG form the basis of home pregnancy tests.
  2. Antibodies against cocaine are used for drug screening and detoxification.
  3. Antibodies against PCP are used for detoxification.

Antibody-Antigen Interations (e.g. prostate specific antigen, PSA):

- Antigen is immunogenic.
- Antibodies generally of very high affinity (nM dissociation constants).
- **Epitope**: region on the antigen that contacts the antibody.

- Interactions between antibody and antigen:
  1. Utilizes all 6 CDRs.
  2. Involves extensive surface contacts between relatively flat surfaces on both the antibody and the immunoglobulin
  3. Mediated by ion-pairing, hydrogen bonds (often mediated by water), van der Waals interactions, hydrophobic interactions.
  4. Usually involve discontinuous segments of the polypeptide chain.
  5. Often highly sensitive to changing one or more residues within the epitope, leading to a distinction between structural and energetic residues within the epitope.

Structural Comparisons of Classes of Immunoglobulins:

Property - Isotype	**IgG1**	**IgG2**	**IgG3**	**IgG4**	**IgA1/ A2**	**IgM**	**IgE**	**IgD**
Structural aspects	Hinge Variant	Hinge Variant	Hinge Variant	Hinge Variant	Forms dimers with J chain	Hinge replaced by $C_\mu 2$	Hinge replaced by Cε2	Has Hinge
Polymeric	No	No	No	No	Dimer-tetramer (S-S bond to J chain)	Pentameric (S-S bonds to adjacent IgM and to J chain)*	No	No
Serum ½ life	23	23	8	23	6	5	2.5	3
Activates Complement	+	+/-	++	-	-	+++	-	-
Crosses Placenta	+	+/-	+	+	-	-	-	-
Binds to Fc receptors on macrophages	++	+/-	++	+	-	+	-	-
Present in Secreations/Milk	-	-	-	-	++ (15g/day)	+	-	-
Histamine release from Mast Cells	-	-	-	-	-	-	+	-
Present in Colostrum	+	+	+	+	-	-	-	-

Immunoglobulins and Development:

- Fetal synthesis of IgM and IgA begin during the 5th month.
- Immature B-cells express IgM on surface. IgM is monomeric on surface.
- Mature B-cells express IgM and IgD on surface.
- Activated B-cells switch class to IgM+IgD, IgA, IgE, IgG, or IgA & IgM, IgE & IgM, IgG & IgM, largely membrane bound.
- Plasma cells can secreate IgM, IgG, IgA, IgE (all but IgD)
- Memory Cells display IgG, IgA, IgE, alone or combined with IgM. These are usually higher affinity than the orginal B-cell clone because of affinity maturation via somatic cell mutation.