ANTIBODY STRUCTURE

reminder: each B cell has unique antibody (Ig)
in resting B cell this is on surface
antigen recognition causes clonal selection and differentiation into plasma cell that produces same Ig in secreted form (Fig. 4.1)

1. Construction of Ig: heavy and light chains
- Ig’s are Y shaped proteins composed of four peptide chains: 2 identical light (L) chains and 2 identical heavy (H) chains.
- Disulfide bonds: The two heavy chains are connected to each other by disulfide bonds, and each light chain is connected to a heavy chain by disulfide bonds. Fig. 4.2

Ig has two functional regions: variable and constant regions (Fig. 4.2).

i) Variable region
- The region that contains the antigen-recognition surface is called variable region.
- Variable region is called such because this region is different in all antibodies. This is because V regions are encoded by recombination of gene segments (lecture 7).

ii) Constant region
- Composed of the constant parts of heavy and light chains (all but the most N-terminal Ig domain). Fig. 4.2. In other words, no gene rearrangement is involved in producing the coding sequence.
- Constant region of heavy chain engages in the effector function (e.g. complement fix).
- Constant region actually be one of five isotypes listed below.

2. Ab structure defined by peptide cleavage (Fig. 4.3)
   **Fab** and **Fc** fragments: Fragment Antigen Binding and Fragment Crystallizable generated by cleavage with the protease papain
   Fc receptors recognize areas of the constant regions of specific Ig isotypes

   - IgG is flexible near hinge (Fig. 4.4), allowing recognition by both arms of epitopes with different spacing.

3. Isotypes
   i) Heavy chain
   - There are five classes of heavy chains: \( \gamma, \alpha, \delta, \mu \) and \( \epsilon \). Encoded by adjacent gene segments.
   - These heavy chains define the isotype of a given antibody: IgG, IgA, IgD, IgM, and IgE. (Fig. 4.5)
   - All five chains have different protein structure and therefore different "effector" function. There are four subtypes of IgG. B cells can change Ab class without changing Ag specificity by a process known as isotype switching.
   - Each class of heavy chain can be expressed in either transmembrane or soluble (secreted) form.
ii) Light chain
- There are two types of light chain; κ and λ
- They are encoded on different chromosomes, but there are no functional differences.
- Either κ or λ can be found on a given antibody as light chain.

4. Tertiary protein structure of Ig
- Abs are made of discrete domains called "immunoglobulin domain", which is approx. 110 amino acids in length and form antiparallel β-sheets held together by DS bonds (Figs. 4.6, 4.7).
- Light chain has two Ig domains; Heavy chain has 4-5 Ig domains.
- The variable region is composed of the N-terminal Ig domain of both the heavy chain (V<sub>H</sub>) and light chain (V<sub>L</sub>).
- Many proteins (not just antibodies) have such domain structure - Ig superfamily. The main protein interaction surfaces are provided by the loops connecting the beta strands. The core structure is highly stable and protease resistant, which probably accounts for its success as a domain in diverse proteins.

- Amino acid sequencing of variable regions of both heavy and light chains shows three regions of hyper-variability which are termed CDR (complementarity-determining regions = hypervariable regions; other portions are framework regions): the three CDR loops of each chain are found at the tip of each arm of the Ab and form antigen binding site (Fig. 4.8).

4. Antibody-antigen interaction
   binding forces
   - electrostatic interactions (ionic; opposite charges)
   - hydrogen bonds
   - Van der Waals forces (short range electron cloud effects)
   - hydrophobic forces (excluding water can be energetically favorable and allow VDW)
   - different antibodies to the same antigen can have different affinities depending on the strength of these binding forces. In general, effective antibodies have very high affinity and don’t easily let go of the Ag.

   binding sites
   - the part of antigen that binds Ab is called epitope
   - usually a protein or carbohydrate structure on the surface of a pathogen (Fig. 4.9)
   - antigens usually stimulate a response better if they are multivalent; (Fig. 4.10) having multiple epitopes or multiple adjacent copies of the same epitope

   - the region of the V<sub>H</sub> + V<sub>L</sub> that contacts epitope can be different in shape and size (Fig. 4.11)

   - epitopes can be linear or discontinuous (conformational) (Fig. 4.12)
Monoclonal antibody (mAb) production (Fig. 4.13)
- Kohler and Milstein in 1975 – Nobel prize in 1984
- Method of immortalizing B cells producing antibodies of known epitope specificity.
- Many applications in basic research, clinical medicine → can read more about medical applications in textbook, last section of chapter (and Fig. 4.15)
- Uses fusion of two different cell types
  1. Mice are immunized with a specific Ag.
  2. Spleen cells are isolated from the mice. Some percentage of these cells will be B cells producing Ab to the Ag. The point is to immortalize B cells from the spleen and then select for the ones specific for the Ag.
  3. Spleen cells are fused with mutant myeloma cell line that is unable to grow in "HAT" selection medium because it lacks certain enzymes. It does not produce Ab on its own.
  4. Fused cells are serial-diluted and cultured in HAT medium, which will allow only the fused cells to grow. Unfused myeloma cells will not be able to grow in HAT, and unfused (mortal) B cells will naturally die out.
  5. Hybridomas will survive.
  6. Identification of the B cell clones that generate Ab specific to the Ag

- Polyclonal antibodies are purified from serum of immunized animals and are derived from multiple different activated B cell clones producing unique antibody structures. Sometimes called antisera (plural of antiserum).

Flow cytometry (Fig. 4.14)
Also known as fluorescence-activated cell sorting, or FACS
- Cells are mixed with antibodies that recognize cell surface molecules (e.g. IgM, TCR)
- Antibodies are linked to fluorescent dyes
- Labeled cell mixture is passed through laser path in FACS, which records fluorescence of each cell in mixture, as well as cell size and shape
- FACS allows the simultaneous measurement of surface expression of multiple proteins
- Can graph data for single antibodies using a histogram showing the distribution of fluorescence intensities across a population; or graph data for two antibodies using a dot plot (each dot represents one cell). Not just a “yes” or “no” technique---obtain quantitative data about expression levels.