CHAPTER 4

Antibody Structure, and the Generation of B-cell Diversity

Recap of chapter 3

• What are the differences between the innate and adaptive immune system?
• In which way are the epitopes of B and T cells different?

Antigen recognition by B cells

Structure of Immunoglobulin G

Function antibody:
• recognition pathogens by variable regions (combination VH & VL)
• recruitment cells immune system for removal tagged pathogens by constant regions (CH)
Structure and function Immunoglobulin fragments
- Cleavage with papain generates Fab (Fragment antigen binding) and Fc (Fragment crystallizable)
- Differences in heavy chain C regions define five isotypes: IgG, IgM, IgD, IgA and IgE

Flexibility of the Ig structure is important!
- V- and C-domains have immunoglobulin fold
- Characterized by antiparallel strands forming two β sheets (sandwich)
- Three hypervariable regions in V-domain form loops contacting antigen

Hypervariable regions make up antigen binding loops
- Variability plot reveals hypervariable sequences of V domains
- Hypervariable (HV) or complementarity determining regions (CDR) are flanked by framework regions (FR)
- FR responsible for immunoglobulin fold
- CDR form loops at exposed side of antibody
Antigen binding sites

- Part of antigen recognized by antibody is called antigen determinant or epitope.
- Antibodies can bind avidly and therefore strongly to repeated epitopes.
- Epitopes consist of a linear or of a discontinuous sequence (linear vs conformational epitope).

Shapes of epitopes

- Type (1): end of polypeptide or polysaccharide binds into pocket formed between VH and VL.
- Type (2): linear epitopes bind into shallower clefts formed by all opposing CDRs of VH and VL.
- Type (3): conformational epitopes often interact via large surface.
- Type (4): pocket within antigen interacts with protruding CDR.

Genomic organization human heavy & light chain locus

- Light chain variable domain is product of rearranged Vl/k and Jl/k.
- Heavy chain variable domain contains rearranged VH, DH and JH.
- CDR3 is formed by fusion of V, (D) and J segment, therefore important in target binding.
Somatic recombination

- Recombination of V1 and J by looping out a DNA segment
- Annealing of Recombination Signal Sequences (RSS) and catalyzed by RAG's
- Diversity determined by number of possible combinations of V and J for VL and V, D and J for VH and number of combinations of VH and VL
- Allelic exclusion prevents recombination of locus on 2nd chromosome: one B cell only produces one antibody

IgM / IgD is first expressed isotype

- Rearranged heavy chain V domain in juxtaposition of Cm / Cd cluster
- Alternative splicing leads to IgM (right figure) or IgD mRNA
- Expression on cell surface via Membrane Coding (MC) exon or secreted (no MC)
- IgM/IgD is first format to contact target
- IgM secreted in high quantities and has effector functions (protective immunity); IgD low levels, no effector functions
Affinity maturation by somatic hypermutations

- IgM has low affinity binding; high avidity (5x2 binding units) compensates for low affinity of binding unit
- During B cell development affinity improved by somatic hypermutation
- Random mutations introduced in V gene (left), but those giving better affinity (and therefore targeting CDR loops) are selected (below)
- Somatic mutation increases affinity, therefore lower degree of avidity needed: isotype switch from IgM (pentamer) to other types (IgG, IgA or IgE; bivalent)

Isotype switching

- Isotype switching ensures fusion of VH to other heavy chain isotype (no change in epitope recognition)
- Isotype light chain not changed
- Initiated by Switch (S) sequences and catalyzed by activation-induced cytidine deaminase (AID)
- Looping out of DNA puts VH in juxtaposition of new isotype

Functions of isotypes

- Neutralization (e.g. blocking interaction virus with cellular receptor)
- Opsonization / activation complement system leading to lysis pathogen and ingestion by phagocytes
- Activation NK cells by binding of Fc to Fc receptors
- Activation mast cells by interaction with IgE receptor

(discussed in Chapter 9)

Properties of isotypes

- Transport across epithelium: secretion into lumen of gut, milk, saliva, sweat and tears to combat parasites, microbes and viruses outside the body
- Transport across placenta to supply fetus with protective antibodies
- Diffusion in extravascular sites of damaged or infected tissues (related to size of antibody)