Chapter 4:
Antibody structure and the generation of B cell diversity

B cells recognize their antigen without needing an antigen presenting cell

Antigen recognition by B cells

Structure of Immunoglobulins

Function antibody:
- recognition pathogens by variable regions (combination VH & VL)
- recruitment cells immune system for removal tagged pathogens by constant regions
**Structure and function Immunoglobulin fragments**

- Cleavage with papain generates Fab (Fragment antigen binding) and Fc (Fragment crystallizable)

**Different Immunoglobulins**

Differences in heavy chain C regions define five isotypes: IgG, IgM, IgD, IgA and IgE => functional difference

Two different light chains: kappa (κ, 2/3) and lambda (λ, 1/3)

= > No functional difference

**Flexibility of the Ig structure is important!**

Movie: Zhang et al, 2015 Scientific Reports

- VH and VL form two hands held together with CDR loops resembling the fingers
- IgG has two arms capable of binding avidly to multivalent targets or repetitive epitopes within a single target
- Hinge has "linear" structure (less stable), but gives flexibility to binding arms enabling avid binding

**Structure of variable region**

- V- and C-domains are characterized by antiparallel strands forming two β sheets (sandwich)
- Three hypervariable regions in V-domain form loops contacting antigen: complementary determining regions (CDRs)
Hypervariable regions make up antigen binding loops

- Variability plot reveals hypervariable sequences of V domains
- 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

Monoclonal antibodies

Monoclonal antibodies (mAb) are antibodies that are identical because they were produced by one type of immune cell, all clones of a single parent cell. Given (almost) any substance, it is possible to create monoclonal antibodies that specifically bind to that substance; they can then serve to detect or purify that substance. This has become an important tool in biochemistry, molecular biology and medicine.
Nobel price in 1984

Jerne, Kohler and Milstein for the discovery of the principle for production of monoclonal antibodies

Monoclonal antibody production

Use of monoclonal antibodies in cancer

<table>
<thead>
<tr>
<th>Four types of therapeutic monoclonal antibody</th>
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<tbody>
<tr>
<td><img src="image1" alt="Mouse" /></td>
</tr>
</tbody>
</table>

- **Mouse**
- **Chimeric**
- **Humanized**
- **Human**
Genomic organization human heavy & light chain locus

- Light chain variable domain is product of rearranged \( V_L / D_L / J_L \) and \( J_L / C_L \)
- Heavy chain variable domain contains rearranged \( V_H / D_H / J_H \)
- CDR3 is formed by fusion of \( V_H, D_H \) and \( J_H \) segment, therefore important in target binding
- Lambda light chain locus contains 30 \( V_L \) and 4 \( J_L \) clusters, kappa 35 \( V_L \), 5 \( J_L \) and 1 \( C_L \), and heavy chain locus 40 \( V_H \), 23 \( D_H \) and 63 followed by 9 \( C_H \) isotypes

VDJ RECOMBINATION

Janeway’s Immunobiology: 5.1

- Recombination of \( V_L \) and \( J_L \) 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 \( V_L \) and \( J_L \) for \( VL \) and \( V_H, D_H \) and \( J_H \) 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
The RAG genes were key elements in the origin of adaptive immunity. RAG genes lack introns and resemble the transposase gene of transposons. Important for function: Recombination process results in an excision circle rather than a linear (and potentially harmful) element.

Junctional diversity
- Fusion of V and J imprecise, i.e. reading frame can be correct or incorrect (leading to a non-functional antibody)
- More variability possible in joining of DH-JH and VH-DH
- P-nucleotides added due to nicking, N-nucleotides by enzyme TdT
- Contribution P- and N-nucleotides is called junctional diversity
- Large diversity of antibodies generated by somatic recombination, VH-VL combinations and junctional diversity

How many different antigen binding sites are possible?

IgM / IgD is first expressed isotype
- Rearranged heavy chain V domain in juxtaposition of Cδ / Cε cluster
- Alternative splicing leads to IgM (right figure) or IgD mRNA
- IgM secreted in high quantities and has effector functions (protective immunity); IgD low levels, no effector functions?
Heavy chain isotypes

- After successful rearrangement VH and VL low affinity antibody secreted in form of pentameric IgM; high avidity (5x2 binding units) compensates for low affinity of binding unit
- Somatic mutation increases affinity, therefore lower degree of avidity needed: isotype switch from IgM (pentamer) to other types (IgG, IgA or IgE; bivalent)

Functions of isotypes

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

<table>
<thead>
<tr>
<th>Property</th>
<th>IgM</th>
<th>IgG1</th>
<th>IgG4</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgA</th>
<th>IgD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport across epithelium</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>-</td>
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<tr>
<td>Transport across placenta</td>
<td>-</td>
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<td>-</td>
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<td>-</td>
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<tr>
<td>Diffusion into extravascular sites</td>
<td>-/+</td>
<td>-+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<tr>
<td>Mean serum level (mg/mL)</td>
<td>1.5</td>
<td>0.05</td>
<td>9</td>
<td>3</td>
<td>1</td>
<td>0.5</td>
<td>2.5</td>
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Factors Influencing Serum Concentrations of Immunoglobulin D in the Adult Population: An Observational Study in Spain

- IgM has low affinity binding
- 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)

Affinity maturation by somatic hypermutations

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Scandinavian Journal of Immunology

Movie by Jullian Kirk Elleker