Types, production of antibodies and antibody/antigen interaction
Antibodies

- Secreted by B lymphocytes
- Great diversity and specificity: >10^9 different antibodies; can distinguish between very similar molecules
- Tag particles for clearance/destruction
- Protect against re-infection (vaccines)
CELLS OF THE IMMUNE SYSTEM
Antibody Structure

**Ig domain**: 110 amino acids; globular domain used in many proteins.

**Variable domains, Constant domains, Hinge.**

**Fab**: fragment antigen binding

**Fc**: fragment crystallizable (effector functions)
Immunoglobulins (Ig) are glycoproteins made up of light (L) and heavy (H) polypeptide chains. The simplest antibody molecule has a Y shape and consists of four polypeptide chains: two H chains and two L chains. The four chains are linked by disulfide bonds.
L and H chains are subdivided into **variable** and **constant** regions. The regions are composed of three-dimensionally folded, repeating segments called domains. An L chain consists of one variable (VL) and one constant (CL) domain. Most H chains consist of one variable (VH) and three constant (CH) domains. (IgG and IgA have three CH domains, whereas IgM and IgE have four.)
The various regions are responsible for antigen binding, whereas the constant regions are responsible for various biologic functions, e.g., complement activation and binding to cell surface receptors.
Human Immunoglobulin
Light Chain Types

Kappa (∩)
Lambda (∩)

one type is found in Ig.
Structure of the Variable Region

- Hypervariable (HVR) or complementarity determining regions (CDR)

Framework regions
Generation of Antibody Diversity

κ light chains: $40 \text{ V}_\kappa \times 5 \text{ J}_\kappa = 200$

λ light chains: $30 \text{ V}_\lambda \times 4 \text{ J}_\lambda = 120$

H chains: $40 \text{ VH} \times 27 \text{ DH} \times 6 \text{ JH} = 6,480$

320 L chains x 6,480 H chains = $2.1 \times 10^6$

Junctional diversity (addition or deletion of nucleotides at recombination sites, especially of H chain), estimated to add $3 \times 10^7$ fold to overall diversity.
When a B cell expands into a clone, it may switch its Ig class. When this happens, the variable region of the antibody stays the same, but the constant region changes.
Antibody Classes: Structure

(a) IgG, IgD
monomeric IgA

(b) IgE and IgM

(d) IgM pentamer

(c) IgA dimer
J chain
secretory component
### Major functional properties of antibodies

<table>
<thead>
<tr>
<th>Antibody class</th>
<th>Major Functional properties</th>
</tr>
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<tbody>
<tr>
<td>IgM</td>
<td>complement activation; antigen trapping; antigen receptor of naïve B cells</td>
</tr>
<tr>
<td>IgG</td>
<td>complement activation, phagocytosis, ADCC, transfer of adaptive immunity to offspring, regulation of antibody production</td>
</tr>
<tr>
<td>IgA</td>
<td>mucosal immunity, phagocytosis</td>
</tr>
<tr>
<td>IgE</td>
<td>activation of mast cells, basophils, eosinophils</td>
</tr>
<tr>
<td>IgD</td>
<td>antigen receptor on naïve B cells</td>
</tr>
</tbody>
</table>
**Antigens**

**Epitope:**
Small part of an antigen that interacts with an antibody.

Any given antigen may have several epitopes.

Each epitope is recognized by a different antibody.

**Epitopes: Antigen Regions that Interact with Antibodies**
Non-covalent forces in antibody - antigen interactions

<table>
<thead>
<tr>
<th>Force</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Electrostatic forces</strong></td>
<td>Attraction between opposite charges</td>
</tr>
<tr>
<td><strong>Hydrogen bonds</strong></td>
<td>Hydrogens shared between electronegative atoms</td>
</tr>
<tr>
<td><strong>Van der Waal’s forces</strong></td>
<td>Fluctuations in electron clouds around molecules oppositely polarise neighbouring atoms</td>
</tr>
<tr>
<td><strong>Hydrophobic forces</strong></td>
<td>Hydrophobic groups pack together to exclude water (involves Van der Waal’s forces)</td>
</tr>
</tbody>
</table>
Clonal Selection of B Cells is Caused by Antigenic Stimulation
ANTIBODIES

POLYCLONAL.
- Derived from different B Lymphocytes cell lines
- Batch to Batch variation affecting Ab reactivity & titre
- NOT Powerful tools for clinical diagnostic tests

MONOCLONAL.
- Derived from a single B cell clone
- mAb offer Reproducible, Predictable & Potentially inexhaustible supply of Ab with exquisite specificity
- Enable the development of secure immunoassay systems.
PRODUCTION OF MONOCLONAL ANTIBODY
PRODUCTION OF MONOCLONAL ANTIBODY

HYBRIDOMA TECHNOLOGY

Step 4: - Fusion of Myeloma Cells with Immune Spleen Cells &
Selection of Hybridoma Cells

1. Plating of Cells in HAT selective Medium
2. Scanning of Viable Hybridomas
Monoclonal antibodies used in medicine

Standardized, unlimited amounts of reagents for diagnosis or therapy (human antibodies or “humanized” antibodies can be made).

<table>
<thead>
<tr>
<th>Monoclonal Antibodies Used in Therapies</th>
</tr>
</thead>
<tbody>
<tr>
<td>monoclonal antibody</td>
</tr>
<tr>
<td>trastuzumab</td>
</tr>
<tr>
<td>infliximab</td>
</tr>
<tr>
<td>rituximab</td>
</tr>
<tr>
<td>abciximab</td>
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<tr>
<td>OKT3</td>
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</tbody>
</table>
Applications of Monoclonal Antibodies

- **Diagnostic Applications**
  - Biosensors & Microarrays

- **Therapeutic Applications**
  - Transplant rejection: Muronomab-CD3
  - Cardiovascular disease: Abciximab
  - Cancer: Rituximab
  - Infectious Diseases: Palivizumab
  - Inflammatory disease: Infliximab

- **Clinical Applications**
  - Purification of drugs, Imaging the target

- **Future Applications**
  - Fight against Bioterrorism
thank you