Chapter 4. Immunoglobulin Structure and Function

1. Functional Regions
2. Types of chains
3. Constant & Variable regions
4. Glycoprotein

- Each heavy and light chain is made up of a number of domains (= Ig folding or Ig domains).
- Light chains consist of 2 domains (C and V).
- Heavy chains have 4-5 domains (depending on the class of antibody).
- Each domain is about 110 amino acids in length and contains an intrachain disulfide bond between two cysteines about 60 amino acids apart.

Basic Antibody Structure
- Multiple myeloma = cancerous plasma cells
- Monomer = 150,000
RECAP:
- The Fc region plays NO role in antigen binding.
- Papain breaks antigen molecules into 2 Fab fragments and an Fc fragment.
- Pepsin breaks antibody molecules into an F(ab')2 fragment and a VERY SMALL pFc' fragment.
- Mercaptoethanol treatment results in 2 heavy and 2 light chains
- Complexes of antibodies cross-linked by antigen are called “immune complexes”.

Summary
- Molecule consists of Constant and Variable regions for both Light and Heavy chains (C\text{H}, V\text{H}, C\text{L}, V\text{L})
- Ig molecule made of domains
- Domains ~ 110 aa
- Each antigen-binding site is made up of the N-terminal domain of the heavy and the light chains
- IgM and IgE possess 4 C\text{H} domains (C\text{H}1-C\text{H}4) while IgG, IgA and IgD have 3 C\text{H} domains (C\text{H}1-C\text{H}3). Hinge region is missing.
- Hypervariable regions in the Variable regions of both H and L chains.

-Within the variable domains are three regions of extreme variability.
- These are referred to as the hypervariable regions.
- These regions of the variable domains actually contact the antigen.
- They therefore make up the antigen-binding site.
- These regions are also called the complementarity-determining regions, or CDRs.
Antibody-Mediated Effector Functions

- Binding to Antigen
- OPSONIZATION: FcR in Macrophages and neutrophils
- COMPLEMENT ACTIVATION: IgG and IgM
- ADCC – NK cells through FcR
- CROSSING EPITHELIAL LAYERS – IgA (but also IgM)
- CROSSING PLACENTA - IgG

Fcγ receptors enhance phagocytosis of foreign cells/particles coated with IgG
Antibody made in response to foreign cells (cells/viral particles/bacteria etc) will bind to those cells.
Macrophages (and neutrophils) possess receptors for the Fc region of IgG.
Binding of macrophage Fc receptors to antibody bound to cells/particles facilitates and increases phagocytosis of cells/particles.

ADCC - Antibody-dependent cellular cytotoxicity - mediated by IgG

Antibody made in response to foreign cells (cells/viral particles/bacteria etc) will bind to those cells.
Cells of the innate immune system (neutrophils, eosinophils, macrophages, NK cells) possess receptors for the Fc region of IgG.
These cells bind to antibody on the surface of foreign cells and release lytic compounds → lysis.
Structural Variants of the Basic Immunoglobulin Molecule

Different heavy chains can be used
- There are five major types of heavy chain \( \rightarrow \) five major classes (isotypes) of antibody
- gamma \( \rightarrow \) IgG (in humans 4 subclasses: IgG1, IgG2, IgG3, IgG4)
- mu \( \rightarrow \) IgM
- alpha \( \rightarrow \) IgA (in humans, 2 subclasses: IgA1, IgA2)
- delta \( \rightarrow \) IgD
- epsilon \( \rightarrow \) IgE

The function of antibody varies depending on which heavy chain is used.

Relative abundance in normal serum:

- IgG: 8 - 16 mg/ml
- IgA: 1.4 - 4 mg/ml
- IgM: 0.5 - 2 mg/ml
- IgD: 0.003 - 0.04 mg/ml
- IgE: 17 - 450 ng/ml (<=0.0005 mg/ml)

(a) IgG
- Most abundant in secondary responses
- Crosses placenta (FcRn)
- Complement activation
- Binds to FcR in phagocytes

Crosses placenta
Crosses placenta
Crosses placenta

Complement Activator
Complement Activator
Complement Activator

Fc binding
Fc binding
Fc binding

IgG1
IgG2
IgG3
IgG4

Disulfide bond

- Best Complement activation
- First Ab produced in neonate
- First antibody produced after challenge
- Mucosal transport (to some degree)
- Monomer on B cells
- J chain: polymeric
Dimer in mucosal secretions
- Mucosal transport
- Monomer in circulation
- J chain (polymeric) and Secretory components

**Role of IgE in allergic reactions**
IgE antibodies mediate the immediate hypersensitivity (allergic) reactions that are responsible for symptoms of hay fever, asthma, hives and anaphylactic shock.

IgE binds to Fc receptors on the membranes of blood basophils and tissue mast cells.

Cross-linkage of receptor-bound IgE molecules by antigen (allergen) induces degranulation of basophils and mast cells.

A variety of pharmacologically active mediators present in the granules are released, giving rise to allergic manifestations

**IgD**
- Role unknown
- Present on the surface of MATURE B cells → Marker!!

**SUMMARY**
- IgA and IgM are secreted across epithelial surfaces
- IgG, IgD and IgE can be found only within the body - in serum or lymph.
- IgA and IgM are also found in serum and lymph BUT IN ADDITION can also be found in secretions such as mucous secretions, saliva and tears.
- The IgA and IgM found in external secretions differs from that found in serum by the presence of an additional component referred to as the "secretory component".
- This component is acquired as the IgA or IgM is transported across the epithelial cell barrier.

**Antigenic Determinants on Immunoglobulins**
- Abs are glycoproteins and themselves very immunogenic
- Epitopes on immunoglobulins are divided into:
  - ISOTYPIC
  - ALLOTYPIC
  - IDIOTYPIC
The function of antibody varies depending on which heavy chain is used.

**Constant region determinants that define each antibody class and subclass**

The function of antibody varies depending on which heavy chain is used.

**Allelic variation (Allotypes):** IgG of a particular class may be slightly different between individuals (e.g. variation in the IgG amino acid sequence)

Note: This type of variation has no effect on antibody function.

**RECAP - Sequence variation in antibodies:**

1. Different light changes - no significant functional effect
2. Different heavy chains - very significant functional effect - **isotypic variation**
3. Allelic variation between individuals - no large functional effect - **allotypic variation**
4. Variation in the antigen-binding site - **idiotypic variation**

**B Cell Receptor (BCR):**
- Short cytoplasmic tail (3-28 aa) ....signaling?
- Signaling through a homodimer, Ig-α and Ig-β
- Ig molecule + Ig-α/Ig-β is the BCR
- The homodimer molecule is member of the Ig superfamily group

**Ig Superfamily**

- Divergence from a common gene ancestor coding for 110 aa.
- A member MUST have a “typical” Ig domain or fold 110 aa with an intra chain disulfide bond 50-70 aa apart.
- Most members do not bind Ag!! Then, they must facilitate interaction with surface proteins
- You must know members with roles in: a) immune function, b) Receptor/Signal transduction, and c) Adhesion
Monoclonal Antibodies

- Kohler & Milstein 1975
- Fusion of normal, activated B cell and plasmacytoma (cancerous plasma cell)
- Hybrid: immortal, secrete Ab, hypoxanthine

Plasmacytoma VS B cell

- Plasmacytoma:
  - Cancerous plasma cell (Immortal)
  - Does not secrete Abs
  - Lacks HGPRT
- Normal spleen B cell
  - Limited life span
  - Secretes Abs
  - Possess HGPRT

RESULTS:

- Spleen B cell: Die in culture
- Hybrid: Immortal, secretes Ab, possesses hypoxanthine (HGPRT)
- Plasmacytoma: Lacks HGPRT
Applications?

- Diagnosis
- Research
- Treatment
- Affinity VS Avidity

**Affinity (polyclonal Ab)** = high because of multiple epitopes

**Avidity (monoclonal Ab)** = low affinity but high avidity because of strong epitope-Ab interaction

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**IgG** - Most abundant Ig of internal body fluids (serum, extracellular fluids) - combats microorganisms and toxins within the body tissues.

**IgA** - Most abundant Ig in mucous secretions - protects external surfaces of the body

**IgM** - The first class of antibody produced during an immune response. Present both in internal body fluids and in secretions.

**IgD** - Functions not well defined. Found mostly on the B cell plasma membrane

**IgE** - Increases during parasitic infections. Causes symptoms of allergy.

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