Immunoglobulins (1 of 2)

We’re going to talk about the molecules of the adaptive immune system, we have previously talked about antigens and in this lecture we’re going to talk about the molecules that binds to them, called immunoglobulins (antibodies).

- Each clone of B-cells synthesizes Igs of single specificity for a specific epitope.
  Recall: epitope is the real small part of an antigen that is identified by the adaptive immune system cells.

- B cell receptors (BCRs) are the Igs on B cell surface.

- When B–cells are stimulated, plasma cells secret soluble Igs in blood (in this case they are called antibodies) that non-covalently bind to antigens.

  Humeral immunity: the soluble reaction in blood between antibodies (B-cells products) and antigens. Whereas T-cells are involved in cellular immunity: T-cells bind to antigens that are membrane bound to MHC

- Secreted antibody act on antigens:
  - immobilize them (becomes harmless). (agglutination)
  - “tag” them for destruction and removal by other immune components. (opsonization)

Structure of Igs

Every Igs is made of 4 polypeptides, 2 identical light chains and 2 identical heavy chains. Each light chain has one variable and one constant domain, and each heavy chain has one variable domain and 3-4 constant domains.

Domain is the 3D structure of a polypeptide chain. All of the domains bind together by disulfide bonds to act as one unit. Each domain is made of about 110
amino acids + intrachain disulfide bonds that forms the loops that maintain its structure.

The N-terminal of both variable heavy (VH) and variable light (VL) chains constitute the antigen-binding site which are responsible for the different specificities between antigens.

Hinge region: A specific amino-acids sequence on each heavy chain that are linked together by disulfide bonds. It is rich in proline a.a and contribute to the flexibility of lgs.

**Isotypes of light and heavy chains**

- The light chain is one of 2 types (isotypes): Kappa or lambda, kappa encoded on chromosome 2 and lambda on chromosome 22. Each Ig contains either two lambda or two kappa chains but not one lambda and one kappa
The heavy chain is one of 5 isotypes (all encoded on chromosome 14):

- IgG - gamma (γ) heavy chains
- IgM - mu (μ) heavy chains
- IgA - alpha (α) heavy chains
- IgD - delta (δ) heavy chains
- IgE - epsilon (ε) heavy chains

The class or subclass is determined by the heavy chain isotype:
For example, if one B-cell produces IgM1 for antigen1 and another B-cell produces IgM2 for antigen2, both IgMs have the same constant domains structure but differ in the variable domains.
But if one B-cells secrete IgM for an antigen then the same B-cell secret IgG for the same antigen the variable domains are the same but the constant domains change. (this is called class switching)

- Variable regions differ in their amino acid sequence between the Igs synthesized by different B cells...so called “variable”.
- Each of gamma, delta, and alpha heavy chains contains 3 constant domains (CH1-CH2-CH3) while each of mu and epsilon heavy chains contains 4 constant domains (CH1-CH2-CH3-CH4)...longer and heavier.

*Identifying important parts*

Scientists treated Igs with enzymes to study the function of each part of them. They used two enzymes: Pepsin and Papain

Pepsin: cuts after the hinge region resulting in one part F(ab')2 (Fragment antibody-binding site prime) the two binding sites are identical, and it is called prime because of the extra amino acids sequence.
The remaining fragments will be degraded because they lack disulfide bonds that connect them.

Papain: cuts before the hinge region resulting in Fab and Fc portions.

Fc portion (fragment crystallizable region, the tail region) it is the effective part. Antigens bind to antigen-binding side but the Fc region is the part that bind Fc receptors on phagocytes and stimulates them.

**Classes and isotypes**

- Each B cell produces only 1 heavy chain isotype except unstimulated B cells, express IgD and IgM.
  B-cells express IgM and IgD, when it is stimulated (e.g. acute hepatitis) its first rapid response is releasing of IgM then IgD.

- When secreted (soluble Igs):
  - IgG and IgE: remain monomeric
  - IgM: pentamer
  - IgA: monomer or dimer

*IgD: almost exclusively membrane-bound*
IgM:

- Cell surface-bound monomer or secreted pentamer (5 monomers connected by disulfide bonds, two of them are connected with J chain)
- Most unstimulated B cells display IgM on their surface
- The first Ig produced following antigen stimulation
- Functions:
  - Immobilizing Ag (agglutination)
  - Complement activation
  - It is the most powerful class in these 2 functions (because of the 10 binding sites).

IgG:

- Cell surface-bound or secreted monomer
- 4 IgG subclasses (IgG1, IgG2, IgG3 and IgG4) due to 4 gamma heavy chain subclasses
- The greatest amount of Igs in serum, & the longest half-life
- Able to cross the placenta (maternal protection)
- Functions:
  - Complement activation
  - Opsonization: binds to Fc receptors on phagocytes.

IgA:

- Monomer in serum and dimer in secretions
• Special receptor in basolateral epithelial cell takes the dimer after it was formed using J chain then a portion of this receptor becomes a secretory component of the antibody which provides resistance against enzymes

• 2 isoforms (IgA1 — in blood and upper GI & IgA2 — in lower GI-) due to alpha1 and alpha2

• Daily secretion quantity more than other classes altogether

• Functions:
  - (Secretory): agglutination (immobilization) of antigens preventing them from binding To epithelial cell receptors
  - (Serum): binding to Fc receptors on phagocytes

IgE:

• Relatively low serum concentration

• Most of IgE produced is adsorbed onto mast cells and eosinophils

• Mast cells and basophils have FceRI (Fc epsilon RI)

• Role against parasites (with eosinophils), especially helminthes.

• Responsible for immediate reaction hypersensitivity.
Electrophoresis of human serum

Gel electrophoresis of serum: the separation of proteins based on their electro/physiochemical characteristics (molecular weight and charge) by passing them from a negative to a positive electrode. The most negative charge travels the furthest towards the positive electrode.
Albumin is the most negative and the most numerous.
Other proteins are called globulins (alpha, beta, and gamma), Igs are gamma globulins they are the least negative and the most abundant Ig is IgG.

- **What is the difference between serum and plasma?**
  Blood is composed of RBCs, WBCs and plasma. Plasma is the acellular part of blood. Serum is the coagulated plasma which contains antibodies but not clotting factors (e.g. fibrinogen). Serology is the study of serum and its components.

- **What is the difference between “monoclonal” and “polyclonal”?**
  Monoclonal is a population of antibodies that are clones of one B-cell. Polyclonal is a population of antibodies that are clones of different B-cells.

- **What is the difference between active immunity and passive immunity?**
  Both can be natural or acquired.
  Examples:
  - IgG from a past acute HBV that was healed → natural active immunity.
  - Ags in a vaccine that induced an immune reaction → acquired active.
  - Ready Abs given to a patient (temporal immunity) → acquired passive.
  - Transplacental IgG → natural passive.

**Hybridoma technology** (Hybrid from a tumor)
The purpose of it: Production of monoclonal antibodies.
1. Scientist cultured immortal cancer cells (myeloma)
2. Injection of a mouse with Ags to stimulate B-cells
3. Fusion of the cancer cells with B-cells taken from the mouse’s spleen or lymph nodes that have enzymes necessary for survival in culture media using polyethylene glycol
4. This fusion results in cancer cell with enzymes that enable them to survive in culture media and B-cells that are immortal
5. From the hybrid cells, they detect the B-cell that can produce the required antibody or can bind a specific antigen and culture it to produce monoclonal
antibodies
6. These antibodies are used in research, diagnostic tests (ELISA, ...) and many other things.