HUMORAL IMMUNITY, PART II

Distribution and function of Antibody isotypes

- Most common site of pathogen introduction: respiratory, digestive, and urogenital tract, and damaged skin. Mucosal surface, tissue, and blood all need protective antibodies.
- Different isotypes are found at different sites (Fig. 9.23).
- The same antigen specificity ($V_H$ and $V_L$) can be linked to different isotypes by class switching; thus a single B cell can produce antibodies, all specific for the same antigen, that provide all the protective functions appropriate for each body compartment.

- Each isotype has a different size, distribution, and function (Fig. 4.32).

- IgM: - First Ig to be expressed on B cells, first isotype secreted in immune response.
  - Tend to be lower in affinity. Makes up for it by being a pentamer when secreted. (Fig. 4.29)
  - IgM found in the blood (large size prevents it from crossing into tissues except during inflammation); together with IgG the IgM in the blood is important to prevent blood-borne infection = septicemia
  - Do not have Fc receptors so do not opsonize directly
  - However, potent activator of complement cascade leading to opsonization (see below)
- IgMs are produced even during secondary and subsequent immune responses and after somatic hypermutation, but other isotypes dominate.

- IgG: - Principal isotype in the extracellular fluid. IgG is small enough to diffuse out of the blood vessels into tissues, and is also actively transported by a Fc receptor called FcRB (Fig. 9.21)
  - Maternal-fetal transfer directly through placenta in utero, again via FcRB.
  - IgG is effective in complement fixation and opsonization (esp. IgG1 and IgG3). (Fig. 4.32)
  - IgG can neutralize bacterial toxins.

- IgA: - Principal type in secretion to body cavities.
  - Weak opsonin and a poor complement activator - mostly neutralizing.
  - IgA-secreting plasma cells are found in lamina propria, just under the basement membrane of many surface epithelia.
  - IgA is secreted as a dimer joined by J chain (same as for pentameric IgM). (Fig. 4.33)
  - Major areas of IgA synthesis: gut, respiratory epithelium, lactating breast, tear and salivary glands.
  - Breast milk contains IgA and protects an infant's gut from infection.(Fig. 9.23)
  - IgA in milk and IgG transfer across placenta are examples of passive transfer of immunity.

- dimeric IgA is transported from lamina propria to the surface of epithelium by poly-Ig receptor via transcytosis (Fig. 9.22).
  - Once on the apical surface poly-Ig receptor is enzymatically cleaved and IgA is secreted with a part of the poly-Ig receptor still attached (called secretory component)
  - IgA neutralizes bacterial toxins and can prevent attachment.
  - monomeric IgA can also be made, mainly by plasma cells in lymph nodes and spleen, and can neutralize viruses and toxins in blood and tissues
Antibody production in newborns (Fig. 9.24)
- between 3-12months babies are most susceptible to infection because maternal IgG is lost from the circulation, IgA (milk) from mother is reduced and their adaptive immune system is not yet mature

- IgE: very low levels in blood or extracellular fluid. Mostly bound to mast cell Fce receptors and participates in allergic responses (mast cells are found under skin, mucosa, and along blood vessels).

**Effector functions**

**Neutralization**
Mostly carried out by IgG and IgA, abundant in extracellular spaces and body cavities, respectively.

1. Virus
   - Viral infection can be blocked by neutralizing antibodies.
   - i.e. influenza and hemagglutinin (HA) --binds to certain carbohydrates expressed on epithelial cells of respiratory tract. (Fig. 9.25; recommend reading the figure caption)

2. Bacterial attachment
   - Some bacteria need to attach to epithelial cells (i.e. Gonorrhea) or extracellular matrix (e.g. Strep) in order to infect.
   - IgA can bind and neutralize attachment. (Fig. 9.26)

3. Toxins
   - Many toxins harm by mimicking cellular counterparts
   - Many toxins can harm in very small quantities: important for the Ab to diffuse into the tissue fast, bind toxins rapidly and with high affinity.
   - Neutralization prevents toxin attachment to host cells (Fig. 9.28)
   - Immunization: diphtheria and tetanus toxins are denatured (now called toxoid) and given to infants. Toxoids lack toxic activity but retain the antigenic epitopes and therefore induce an immune response to the toxoid and the native toxin.
   - **Passive immunization**: when there is no time to induce adaptive immunity (snake venom), neutralizing Ab from another organism is injected (horse).

**Complement activation**
- Antibodies can activate the complement cascade, which is called the classical pathway (also refers to activation initiated by C-reactive protein, chapter 2).
- IgG (esp. 1 and 3) and IgM are potent complement activators. (Fig. 9.29)
- C1q does not bind to free pentameric IgM or monomeric IgG. Antibody-antigen binding causes changes in Ab conformation (pentameric IgM → planar to staple form; Fig. 9.30) or Ab clustering (IgG, Fig. 9.34, left), and these can be recognized by C1q.

- the alternative C3 convertase amplifies complement deposition initiated by the classical pathway (Fig. 9.32)

- high affinity IgG can bind to pathogen fragments and activate complement, leading to formation of immune complexes (Fig. 9.34, right)

CR1 is most important for clearing immune complexes from blood

expressed on RBCs

when blood passes through the liver, macrophages strip off the immune complexes from RBC surface (Fig. 9.35)

too much circulating immune complex leads to kidney damage

Opsonization

- **Fc receptors**, which are receptors that bind to the Fc portion of an antibody, are expressed on effector cells. These include macrophages, dendritic cells, granulocytes, mast cells. There are many different Fc receptors, each binding to a different isotype.

  - Different cell types express different sets of Fc receptors.
  - FcγR, FcεR, and FcαR: bind to IgG, IgE and IgA, respectively. (Fig. 9.46)

- Fc receptors belong to the Ig superfamily.

- FcRs are multi-protein complexes (separate Ab binding and signaling components)

- Q: Why don't you get constant activation of effector cells bearing Fc receptors? After all, there are free antibodies circulating in the body at all times.

  A: Only when Abs bind to the surface of a pathogen do you get clustering of Ab. This increases avidity of the Fc receptor-Ab interaction. At the same time clustering (and therefore activation) of FcR occurs. Both of these are required for FcR activation.

Types of effector functions initiated by opsonization:

A. Phagocytosis (Fig. 9.41)

  - Macrophages, monocytes and neutrophils have Fcγ receptors (mainly FcγRI) that can bind opsonized pathogens. Pathogens are ingested into phagosomes, which fuse with lysosomes (now called phagolysosomes). Lysosomes contain hydrolytic enzymes as well as oxygen radicals and nitric oxides, which can all contribute to elimination of the pathogen.

  - What if the pathogen is too big for phagocytosis? In such cases (i.e. parasites) effector cells surround the parasite and releases granular contents directly toward the pathogen. The eosinophil is the most common agent of parasitic immune response. (Fig. 9.45)

  - IgM doesn't opsonize per se (no Fc receptor for IgM) but leads to complement deposition on pathogen; important for clearance of encapsulated bacteria such as *Strep pneumoniae*
B. **ADCC** (antibody-dependent cell-mediated cytotoxicity)
   - NK cells are non-B, non-T lymphoid cells that are capable of direct cell lysis.
   - They express FcγRIII that can recognize opsonized antigens expressed on target cells. Such targets would include virally infected cells that express some viral proteins on cell surface.
   - Opsonization of the target triggers lysis of the cell by NK cells through similar mechanisms to that of CTL. (Fig. 9.43)

C. Mast cells
   - Found in vascularized connective tissues just beneath epithelial layer (including submucosal tissues of gastrointestinal and respiratory tracts). (Fig. 9.23)
   - Contains **histamine** and other granule contents that cause **vasodilation** - results in increased blood flow (and therefore migration of effector cells and factors) to the site of immune response.
   - FcεR has a high affinity for IgE; most IgE in your system is found already bound to FcεR on mast cells and basophils.
   - Antigen causes crosslinking of FcεR and this in turn causes release of granules. (Fig. 9.44)

   - mast cell degranulation critical for immunity to parasites in developing world; for example, mast cell activation in gut leads to expulsion of GI contents. In developed countries where parasites are not common, mast cells and IgE are mainly a nuisance → allergy and asthma