The Acquired Immune System

1. **Characteristics of the immune response. Remember, both arms of the immune system are activated at the same time, it just takes longer for the acquired immune system to be able to respond.**

A. Humoral versus Cellular Immunity: T cells and B cells function and are activated COMPLETELY DIFFERENTLY FROM EACH OTHER.

1) T cells: T cells are said to be part of a cell-mediated response, because they interact directly with cells to function. They have a number of functions in the acquired immune response: inflammatory, helper, cytotoxic and regulatory.
   a. Their receptor is called a T cell receptor or **TCR**.
   b. T cells recognize one MHC molecule and its associated peptide to be able to become activated. It is said therefore, that T cells are restricted by MHC/peptides to become activated.
   c. Each T cell activated will only respond to one TCR/peptide combination.

2) B cells and antibodies -B cells are said to be part of a humoral response, because B cells produce products (antibodies) that are the actual mediators that destroy pathogens. These antibodies are found in the blood and body fluids that used to be known as the body's humors- hence the term humoral response.
   a. B cells can respond to a broader array of antigens than T cells can: lipids, carbohydrates, whole proteins.
   b. However, B cells require help from a T cell response to become fully activated. Therefore, T cells regulate the acquired immune response.
   c. Each B cell activated will produce a specific antibody that is targeted to one pathogenic epitope. However, multiple B cells may be activated during any acquired immune response to a pathogen.

3) Each lymphocyte has a receptor (that is randomly generated) that is UNIQUE to every cell. These receptors are generated and tested during the process of positive and negative selection.
   a. Every B cell has a unique receptor (known as a B cell receptor or **BCR**) and each B cell can only bind to one antigen.
      i. There are hundreds of copies of this unique BCR on a single B cell.
      ii. An antigen for a B cell can be ONE protein, carbohydrate or lipid.
      iii. There are millions of different B cells.

iv. At least two BCRs on one B cell must bind to activate a specific B cell.
v. Once activated, the B cell will become a plasma cell that secretes antibodies. Secreted antibodies are composed of constant regions (responsible for antibody function) and variable regions (responsible for antigen recognition).

b. Every T cell has a unique receptor (known as a T cell receptor or **TCR**) and each T cell can only recognize one antigen.
i. There are hundreds of copies of a unique TCR on a single T cell.
ii. The antigen that a TCR recognizes is ONLY a peptide that has to be displayed for recognition in a specific MHC molecule.
iii. The TCR is composed of two chains that have both constant regions and variable regions (responsible for antigen recognition).
iv. TCRs are never secreted.

2. **Lymphoid system**

A. Blood and circulation-closed system pumped by a heart.

B. Lymphatic system: composed of lymphoid vessels, primary and secondary lymphoid organs.

1) Lymphatic system is open ended. The fluid in the system is pumped by muscle movement.
a. The same fluid has different names in different body compartments: plasma→interstitial fluid→lymph→plasma

b. Lymph is the fluid that circulates throughout the lymphatic system.

c. Filters anything present in body tissues through places where pathogens can be recognized.

2) **Primary lymphoid organs are** where lymphocytes are educated.
a. B cells develop in the **bone marrow**.
b. T cells develop in the **thymus**.

3) **Secondary lymph organs**:
a. **Lymph nodes**-filter lymph. The lymph filters into the lymph node. The T cell region is called the paracortex and the B cell region called the

follicles. Afferent lymphatic vessles bring the lymph bearing antigens/pathogens into the lymph node and Efferent vessels out to the next lymph node.

b. MALT and SALT- mucosal and skin associated lymphoid tissue/lymph nodes. Peyers Patches have B cell follicles and T cell regions.

c. Spleen-filters and monitors the **blood**. There are B cell follicles and the T cell regions are called periarteriolar lymphoid sheaths (PALS).

3. HOW IT ALL WORKS TOGETHER

A. First, the BCR and the TCR must be generated in the primary lymphoid organs and are tested for functionality in positive and negative selection.

1) The process of generating a receptor is done using gene rearrangement (cutting and splicing the chromosome) to create unique **variable** regions that will create unique a TCR or BCR on every lymphocyte. Immature T cells and B cells must then be tested to determine if the random process that generated the receptor has actually created a functional BCR or TCR.
   a. By rearranging these gene cassettes we can create $5x10^{13}$ possible B cells and 10^{18} possible T cells (1,000,000,000,000,000,000- a quintillion T cells).
   b. Each receptor is unique.

2) B and T cells test their receptors to see if they are functional in the primary lymphoid organs. B cells in bone marrow, T cells in thymus.

3) This selection process is called positive and negative selection. Because each receptor is generated individually, each receptor must be tested to make sure it is functional, as well as to ensure it is not EXCESSIVELY self-reactive.

4) Positive selection (this is essentially true for both B and T cells although the process is different for both).
   a. If the lymphocyte receptor can bind and send a signal to the nucleus, this means the receptor works, and the lymphocytes are allowed to migrate out of the primary lymphatic organs.
   b. However, if they cannot bind to an antigen and do not send a signal, they are deleted in positive selection.
      i. T cells must bind self MHC molecules/self peptides.

ii. B cells bind whole antigens.

c. The majority of all the T and B cells created undergo apoptosis at this time because their receptors are not functional.

B. Negative selection: if the lymphocytes recognize self too strongly, they may be auto-reactive and dangerous to self. Therefore, if a receptor on a cell binds self too strongly, negative selection occurs. Two things can happen:
1) The cell is deleted.
2) It becomes a regulatory cell that inhibits auto-reactive immune responses.

C. Lymphocytes that have matured in positive and negative selection migrate to the **secondary lymphoid organs**. These are lymphocytes that have never been activated are called MATURE NAÏVE cells.

1) The FIRST time a pathogen is encountered, lymphocytes MUST be activated by **Signal 1** and **Signal 2**.

2) Signal 1 and signal 2 are different for naïve T and naïve B cells.

D. Activation induces the lymphocytes to multiply. These create a larger population of activated cells, or a cloned army of activated cells.
1) Activated T cells become **EFFECTOR CELLS**

2) Activated B cells become **PLASMA CELLS**.

3) Once activated, both B and T cells have cells that also divide and multiply, but don't become activated. These clones are held back for subsequent infections and become **MEMORY CELLS** instead of becoming effector T or plasma B cells.

E. Activated lymphocytes migrate out to destroy the pathogen. T cells and B cells have different functions. What they do/produce depends on their activating signal from the APC.
1) Plasma cells start to make antibodies.

2) T cells migrate to the site of infection to perform T cell duties.

F. The memory cells wait for the next time THAT SAME pathogen is encountered and will be able to RESPOND more rapidly than naïve cells can. They also no longer need APCs to tell them what to target since they have already been activated once.

4. How to activate T cells:

A. Signal One is the recognition of MHC/peptide antigen by a unique TCR/T cell. Every T cell has a CO-RECEPTOR that is common between classes of T cells. The co-receptor helps stabilize and signal the interaction between a TCR and a MHC molecule
   1) CD8 is the co-receptor for T cytotoxic cells
   2) CD4 is the co-receptor for T helper cells.

B. A naïve T cell MUST receive two signals to become activated. **Signal 1 alone will anergize or turn off the T cell. All T cells are turned on in the same way:**

   1) **Signal 1** is binding to the correct APC"s MHC molecule and peptide the T cell recognizes plus co-receptor interaction.
      a. T helper cells can ONLY bind to MHC class II/peptide.
      b. T cytotoxic cells can ONLY bind to MHC class I/peptide.

   2) **Signal 2** is a second set of ligands/receptors that must bind to send an activation signal into the cell that is being activated. The second signal for ALL T CELLS IS THE SAME.
      a. **The B7 molecule on an APC must bind to the CD28 receptor on a T cell**.
      b. Without this second signal, a naive T cell CANNOT BECOME ACTIVATED. It will be turned off or anergized.

C. Once the naïve T cell has become activated, it is called an effector T cell.

D. Some activated T cells are destined to become memory T cells that are important in creating a memory response to a specific pathogen.

E. **T helper (T_H) cells**
   1) Function: T_H cells are responsible for telling the cells of the immune system what they need to do. They are responsible for orchestrating the immune response.

2) Naïve T_H cell activation
   a. Only activated APCs can activate a naïve T cell to become an effector T cell.
   b. Before activation all T_H have a T_H0 phenotype. This means that what type of T helper cell they will become has not been established yet.
      i. **Signal 1**: binding of TCR to a unique combination of MHC class II/peptide molecules on an APC. CD4 helps stabilize this interaction.
      ii. **Signal 2**: B7 molecule on the APC → CD28 receptor on the T cell.
      iii. Without this second signal, a naive T cell CANNOT BECOME ACTIVATED. It will be turned off or anergized.

3) Activated effector T_H can produce a number of different cytokines.
   a. **T_H** 1 induces a cytolytic immune response:
      i. Activate T_c cells by producing IL-2 (T cell growth factor)
      ii. Activate macrophages by producing IFN-γ
   b. **T_H** 2 cells induce a humoral response: T_H 2 cells activate B cells by producing pro-antibody production cytokines such as IL-4, IL-5, IL-13
   c. **T_H** 17 cells induce an inflammatory response by producing IL-17 which act on neutrophils.
   d. **T_H** Reg cells have been activated in negative selection and are responsible for producing cytokines that shut down an immune response (IL-10, TGF-β)

F. Some activated **T_H** cells will clonally expand into memory cells instead of effector cells so they will be ready for the next encounter with this specific pathogen

5. **T cytotoxic (T_C) cells**

A. T_C activation
   1) Naïve T_C cell activation
      a. Only activated APCs can activate a naïve T cell to become an effector T cell.
      b. Before activation all T_C are mature naïve cells.
         i. Signal 1: binding of TCR to a unique combination of MHC class I/peptide molecules on an APC. CD8 helps stabilize this interaction.
         ii. Signal 2: B7 molecule on the APC → CD28 receptor on the T cell.
         iii. Without this second signal, a naive T cell CANNOT BECOME ACTIVATED. It will be turned off or anergized.

B. Some activated Tc cells will differentiate into memory Tc cells and will be ready for the next encounter with this specific pathogen.

C. The effector T_C cells then are responsible for going out and finding the same MHC class I/peptide on infected cells and killing them. Memory T_C wait for the next exposure to the pathogen to become activated.

1) Effector T_C cells produce granzymes and perforins to destroy target cells.
   a. Perforins are similar to complement MAC and form pores in the target cell.
   b. Granzymes are enzymes designed to induce the cell to under apoptosis and enter the cell through the pores created by the perforins.

2) Following activation both memory T_C and T_H cells ONLY require Signal 1 (MHC/peptide→TCR interaction) to become activated. They do not require Signal 2.

6. **Natural Killer (NK) cells**

Are large granular **lymphocytes** that are not T or B cells. They are a lymphocyte that is part of the **innate immune response**. NK cells are automatic killers.

A. They have two functions to play in the immune response

1) They kill aberrant cells that may be virally infected or cancerous. Some virally infected or tumor cells can repressed the expression of MHC class I molecules on the surface of a cell. NK cells look for nucleated cells that DON'T HAVE MHC class I molecules on their surface and kill them with perforin and granzyme. They are the yin to the Tc cell yang.

2) They also play a role in humoral responses. They have specialized antibody receptors that bind to antibody coated targets through **ADCC** that destroys parasites and helminths.

7. B cells activation is completely different from T cell activation!!!

A. A mature naïve B cell must be activated by an effector T helper 2 cell.

1) Signal 1 for a B cell: Two BCRs bound by an antigen to create movement on the surface of the B cell. This is also called cross-linking of the BCR by antigen. The physical movement of the two receptors sends an activation signal into the B cell.

a. This PARTIALLY activates the B cell and up-regulates a molecule called CD40.
b. The B cell starts dividing (only a little), makes some IgM and goes looking for T cell help.

2) Signal 2 comes from an activated effector T_H cell (The T helper cell was activated by an activated APC). This is called a T dependent response because it requires the second signal only an activated T helper cell can provide.
   a. The effector T_H2 cell finds the **semi**-activated B cell and gives it the second signal necessary for the B cell to become **fully** activated.

   b. This is CD40L (on the T cell) binding to CD40 on the B cell. This sends the signal that the B cell is fully activated.

   c. Then, cytokines also produced by the T_H 2 cell (especially IL-4, IL-5 and IL-13) activate and instruct the B cell as to what class of antibody the B cell clones need to switch to for a response to this particular pathogen. Class switching occurs as the clonal expansion takes place, as the B cells divide and replicate their DNA

   d. Once they start clonally expanding this new class of antibody will be used as the BCR on that cell. Each clone of the B cell will only be able to make the isotype of antibody that the cell has switched (it will switch to IgA/IgG/IgE)

   e. Once it has received the second signal from an effector Th2 cell, the clonal offspring of the fully activated B cell will generate a BCR that becomes better at binding antigen, by a process called affinity maturation.

3) The hallmarks of full B cell activation are class switching, affinity maturation and memory cell generation. Without a second signal, a naïve mature B cell cannot do ANY of these things (it can only make a little IgM).

4) Once the pathogen has been eliminated the majority of the plasma cells die and the antibody levels decline.

5) Memory cells are the sentries that stay in the lymph node and wait for the next exposure to the same pathogen. Memory B cells only need Signal one to

make the same isotype of antibody that their original "mother" cell was instructed to make. This means that crosslinking of the receptor on a B cell will cause that cell to secrete antibodies of the correct isotype faster.

B. Secondary response

1) This response is much more intense and occurs more rapidly than that made during first exposure to antigen (primary response).

a. A memory cell no longer requires a second signal from an APC to switch the next time the same pathogen is encountered.

b. The first signal alone –(cross-linking)-is sufficient to induce a naive B cell to start producing IgM antibody, but not to class switch. A memory cell will start producing its specific antibody in the isotype it was told to produce when it was first activated.

8. B cells can act as an APC ONLY for antigens that can bind to its specific BCR. Exactly the same thing happens except the APC is a B cell.

9. Haptens are single epitopes (too small to activate a B cell response- do not crosslink BCRs). Lipids and carbohydrates can partially activate a B cell (signal 1) but cannot activate a T cell response (no signal 2), so these are T independent responses. They CANNOT generate memory, class switch or undergo affinity maturation. A B cell that has only received one signal will only be able to make a little IgM.

Antibodies

1. The job of a B cell is to make antibodies against specific pathogens.
2. What are antibodies? They are proteins that are secreted into the body fluids that bind to identify and eliminate antigens/pathogens.
   A. The BCR on a B cell will bind an identical antigen to what antibody is secreted by that B cell.
      1) Each antibody starts as a monomer
      2) Each antibody monomer is composed of two identical heavy chains and two identical light chains.
      3) Each antibody monomer has a variable region responsible for binding antigen (Fab). The variable region created by the combination of the light chain and heavy chain is unique to each B cell. There is a constant region (Fc) that allows antibodies to have specific functions.

a. The Fab region binds to antigens via non-covalent interactions between the antigen and the variable region.
b. Shape, hydrogen bonding, hydrophobic/hydrophilic, pos/neg charges determine how well an antibody binds.

B. It is the Fc regions that create different classes of antibodies (IgM, IgG, IgE, IgA and IgD). Each of these has specific functions as well a site of action.
   a. These constant regions are the same in all humans.
   b. They are slightly different in different animals. This means that IgG from a mouse "looks" slightly different than what human IgG looks like.
   1) The BCR on all NAÏVE B cells is always both IgM and IgD.
   2) The default antibody <u>always</u> secreted initially by a naïve partially activated B cell is IgM. IgD is never secreted.
   3) On one B cell, the Fab region will be identical although the constant regions are transcripts/translations of either the δ or the μ gene cassette.

C. Class switching: There are 3 possible changes: from μ –––> γ, ε, α. These cassettes are responsible for making a different class of antibody

   1) When a B cell switches constant regions (Fc), the genetic cassettes used to create the **variable region** of both the heavy and light chains will **remain the same**.
   2) When a B cell switches Fc regions, the BCR on the clonal offspring will be the same as the antibody it secretes (secretes IgG, has IgG as its receptor)

2. Antibody function : Functions of antibodies are neutralization, opsonization, complement activation, immobilization, ADCC, and agglutination.

A. **Complement activation**- classical pathway (primarily IgG and IgM activate complement)

B. **Opsonization**- Coating bacteria with antibody (monomers work best for opsonization).

C. **Antibody-dependent cell-mediated cytotoxicity (ADCC)**. Not all antibodies participate in ADCC (only IgE and IgG)

D. Neutralization
   1) Antibody blocks pathogen attachment site (especially good for viruses).
   2) Blocks toxin activity by binding it before it can attach to receptor on cell.

3) Any antibody can neutralize a pathogen/virus/toxin

E. Precipitation

1) Involves soluble antigens.
2) Antibodies bound to antigens precipitates them out of solution, but antigen to antibody ration critical for it to work effectively.

F. Agglutination

1) Antibodies aggregate large particulate cells or molecules.
2) Allow phagocytes to ingest large amounts of bacteria or antigen easily.

G. What can't antibodies do? Antibodies are not effective against intracellular pathogens, such as replicating viruses, or intracellular bacteria (ex. those that hide out in macrophages or other host cells).

2. Classes of antibodies: All antibodies have specific functions that they perform. Not all antibodies can perform every function.

A. **IgM**- 5-10% in serum

1) The only class of antibody that doesn't need T help to be produced. It is the default antibody produced by all naive B cells. It is the BCR for naïve B cells.
2) When secreted by plasma cell it is secreted as a pentamer, joined by J chain .
3) Fixes complement, e.g. activates the classical complement cascade. Because it is a pentamer, is very, very good at activating complement.
4) Neutralizes bacterial toxins.
5) Pentamer is too large to get out of circulation and is primarily found in the blood. IgM cannot cross the placenta.

B. **IgG**-80% in serum

1) Is secreted by plasma cells in monomeric form, activates classical complement cascade when two IgG antibodies bind to the same antigen and C1 binds to the IgG Fc region.
2) Only antibody able to cross the placenta and confer immunity to a fetus (Fig. 16.7) due to an IgGR found on the placenta.
3) Protects against viral and bacterial infection.
4) IgG is the primary antibody against aberrant host cells (ex. infected or cancers cells). NK cells play the primary role in ADCC against IgG-coated cells. NK cells bind to the antibody-coated cells via their receptor for IgG Fc and lob toxic molecules at the infected/cancer cell to destroy it.

C. **IgA**- 10-15% in **serum**
   1) Found in blood as monomer and found in **secretions** (saliva, sweat, mucus) as a dimer, joined by a J chain and a secretory piece.
   2) Transmitted to newborns in breast milk and due to presence in all secretions as well as serum, it is the **most abundant antibody in the overall body**.

D. **IgD**- 0.2% in serum
   1) Found primarily on surface of naive B cells as BCR for naive B cells.
   2) Not secreted into serum.

E. **IgE**- least abundant in serum (bound in tissues by mast cells)
   1) Found in higher concentrations in allergic (ATOPIC) individuals
   2) Mediates response to helminths (parasitic worms).
   3) Is THE major antibody involved in ADCC against helminths. IgE binds to the parasites.
   4) Mast cells, basophils and eosinophils are the primary cells that act in this pathway because they bind to parasite-bound IgE via FcRs found on the surface of the mast cells. This causes the mast cells to degranulate, releasing histamine and other chemicals making life miserable for the helminths!

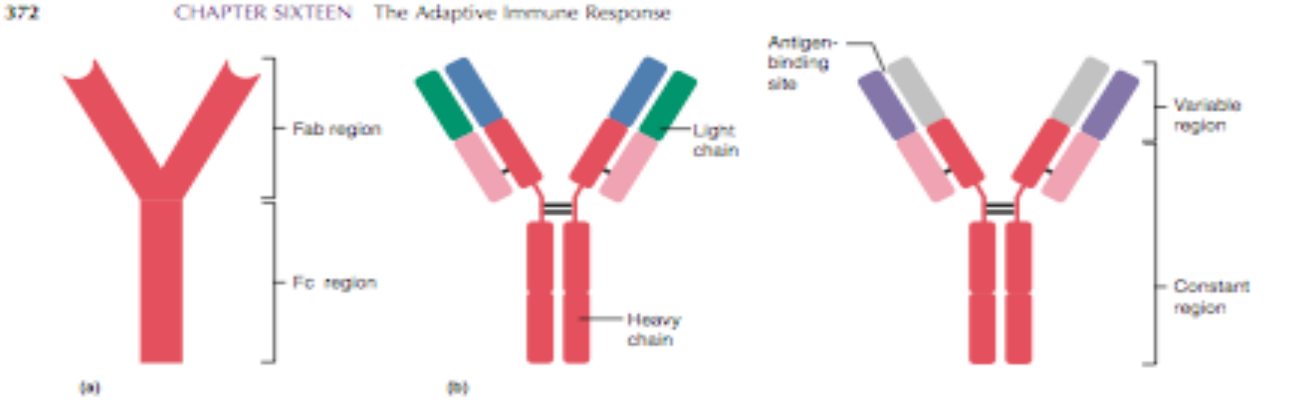

FIGURE 16.5 Basic Structure of an Antibody Molecule (a) The Y-shaped molecule; the arms of the Y make up the Fab regions, and the stem is the Fc region. **(b)** The molecule is made up of two identical heavy chains and two identical light chains. Disulfide bonds join the two chains as well as the two halves of the molecule. The constant region is made up of the regions depicted in shades of red. The variable regions differ among antibody molecules, and account for the antigen-binding specificity of antibody molecules.

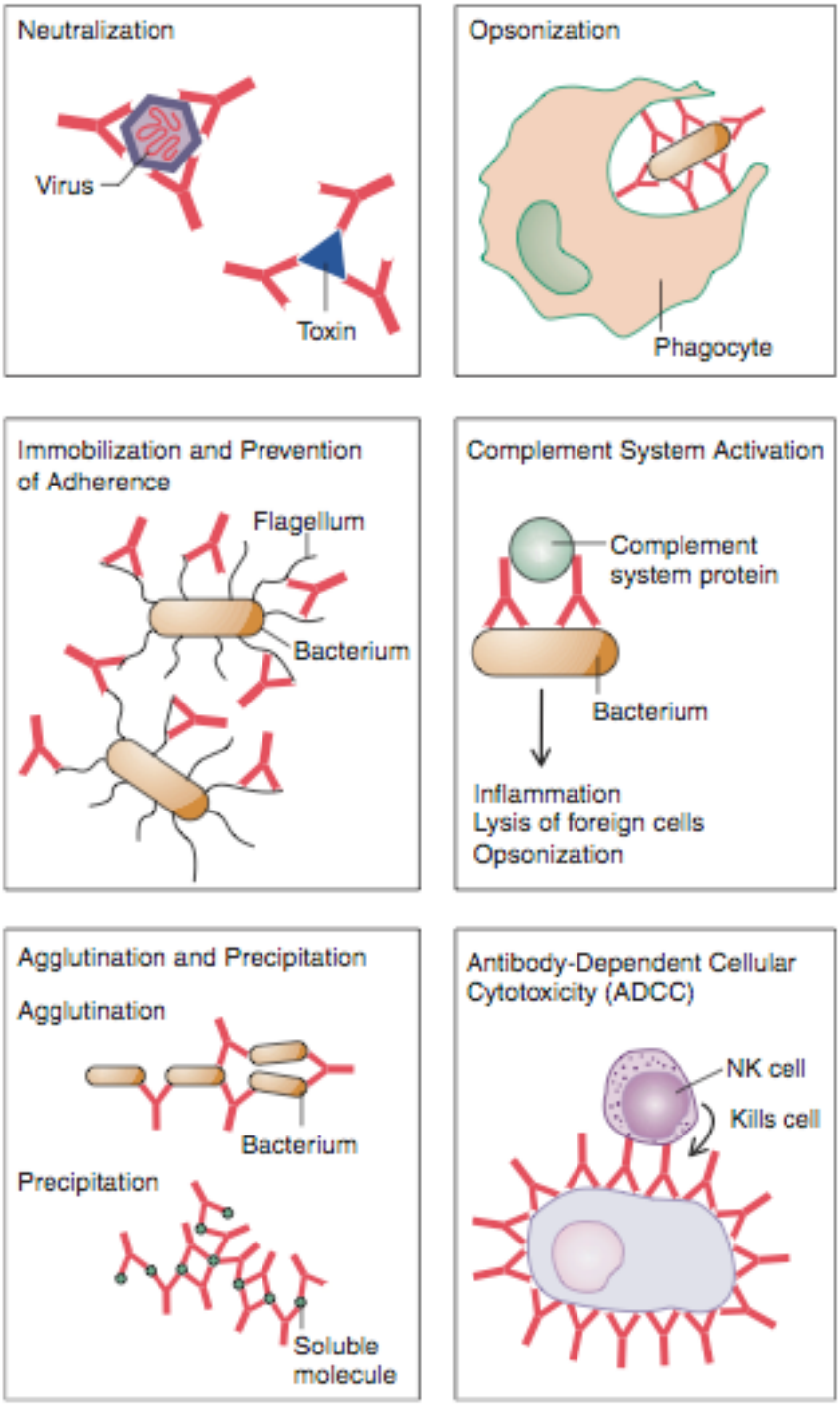

FIGURE 16.6 Protective Outcomes of Antibody-Antigen Binding

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