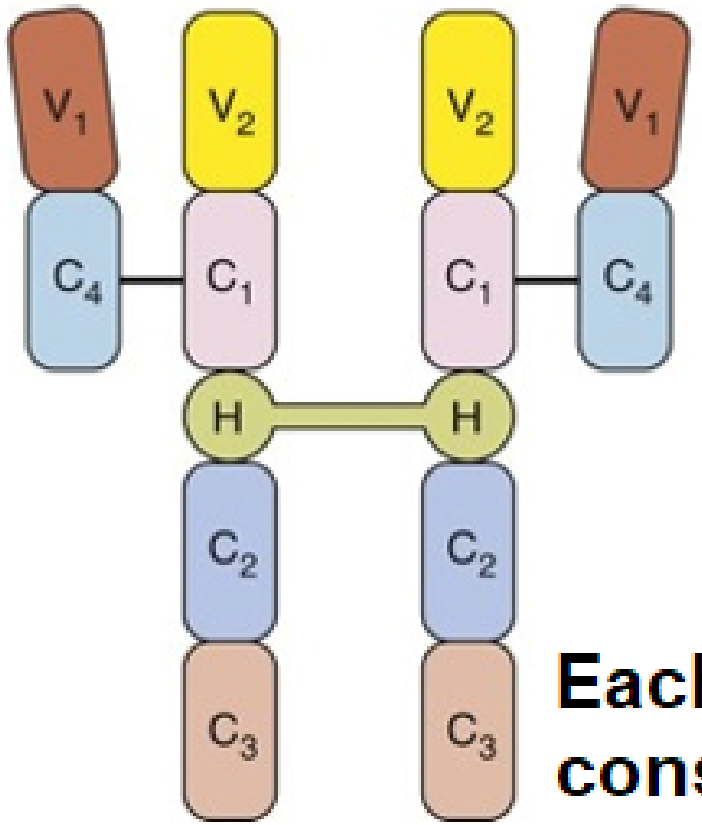
Lecture 3C:
Adaptive Immunity:
Humoral Immune Responses
Excessive Immune Responses

The Adaptive Immune Response: Humoral Immunity

- **BCRs** (B cell receptors) are antigen receptors on the surface of a B lymphocyte. Like TCRs, the 3D shape of the BCR will normally bind only to the 3D shape of one type of antigenic molecule.
  - BCRs are actually a class of **antibody molecule** (class IgD or IgM antibody) . Antibody molecules have a typical **Y-shape** produced by multiple protein subunits.
  - Two classes of genes contribute to each antibody molecule, one for the **variable (V) regions** that make up the antigen binding site, and one for the **constant (C) regions** that are essentially the same for all antibody classes.
  - Rearrangement, recombination and splicing of V region genes allows for the recognition of about $\mathbf{10^{11}}$ different antigenic molecules—many more than the human body encounters! The capacity of **TCRs** is similar, but TCRs aren't antibodies.

The Adaptive Immune Response: Humoral Immunity 1



V=Variable Region

C=Constant Region

H=Hinge Region

Each antigen-binding site consists of two variable regions.

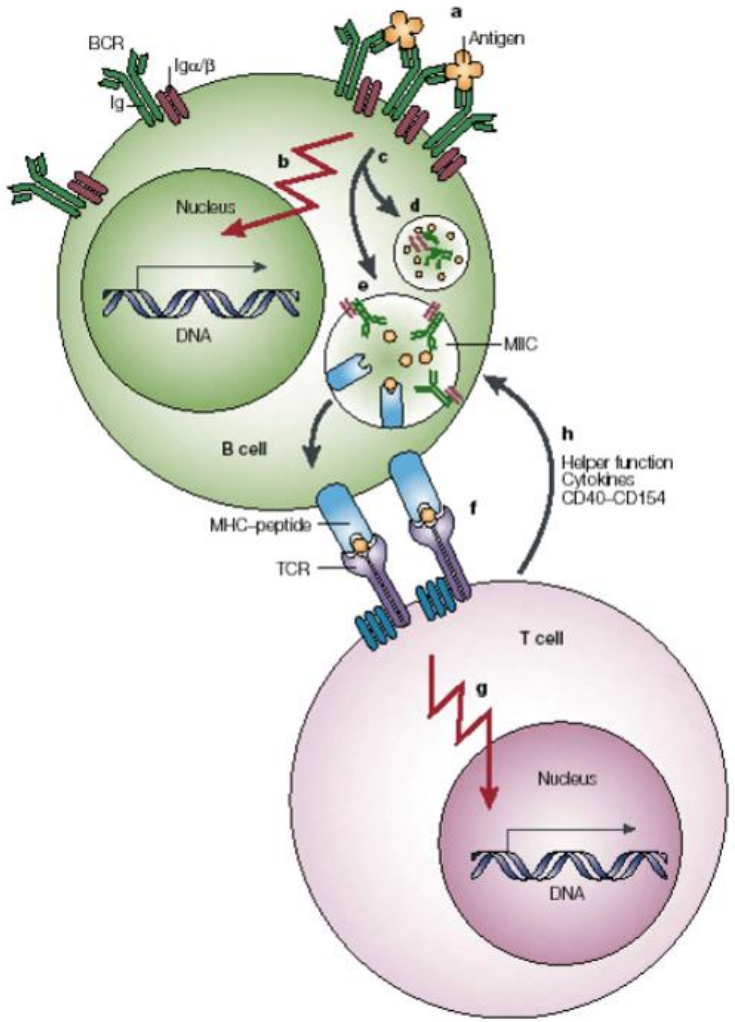
The Adaptive Immune Response: Humoral Immunity 2

- **Activation of a Naïve B cell**
  - Recall that B cells react to **humoral antigens**, those located in extracellular body fluids (plasma, interstitial fluid, etc.)
  - B cell activation requires **"cross-linking" of BCRs**. This means that neighboring cell surface BCRs must **simultaneously** bind to specific antigenic molecules.
  - BCR cross-linking is not sufficient to activate the B cell in most cases. **Effector helper T cell assistance** is usually required for **protein** antigens. B cells present antigens **bound to MHC II** to Helper T cells.
  - First the BCR-antigen binding triggers the B cell to **endocytose the BCR-antigen complex**. The antigen is then **processed into peptides** and displayed with MHC II on the B cell surface.
  - An effector **helper T cell** with the appropriate TCR for the antigen will then bind to the MHC II/antigen complex.

The Adaptive Immune Response: Humoral Immunity 3

- B cell activation requires more than just the main TCR-MHC II-antigenic peptide binding, including costimulation by **B7 to CD28** and **CD154 to CD40** binding. See image on the next slide.
- These surface interactions trigger **cytoplasmic pathways** in both the B cell and the Helper T cell that lead to B cell activation. Certain **cytokines** released by the effector Helper T cell are also required.
- Some B cells have BCRs that bind **non-protein antigens** like microbial surface carbohydrates or lipids. Such antigens are called **T cell-independent antigens**. (T cells only recognize peptide antigens.). B cell activation in this case just requires the cross-linking of B cell receptors (BCRs) by the antigen. Memory cell formation **does not occur** when B cells are activated by T cell-independent antigens.

The Adaptive Immune Response: Humoral Immunity 4



B Cell Activation Process:

1. Endocytosis of BCRs crosslinked by humoral antigen

2. Processing of humoral antigen

3. Display of processed antigen/MHC II complex to Helper T cell

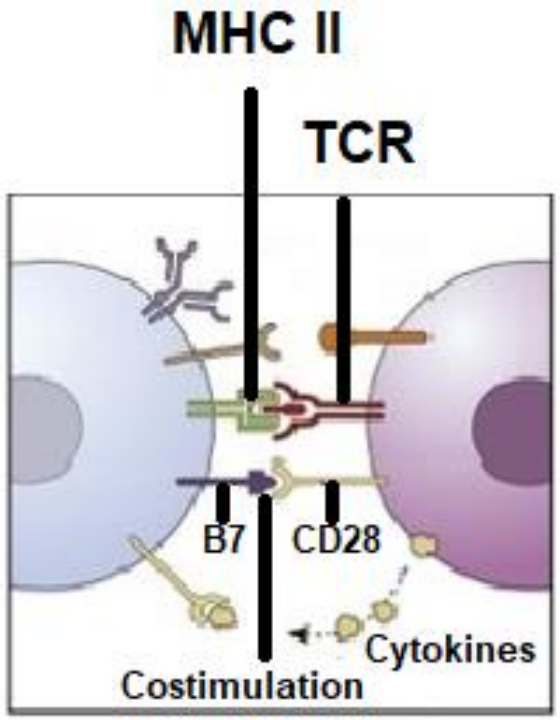
4. Binding of TCR to antigen/MHC II complex

5. Costimulation

6. Release of cytokines from Helper T cell to B cell

7. B cell activation

The Adaptive Immune Response: Humoral Immunity 5



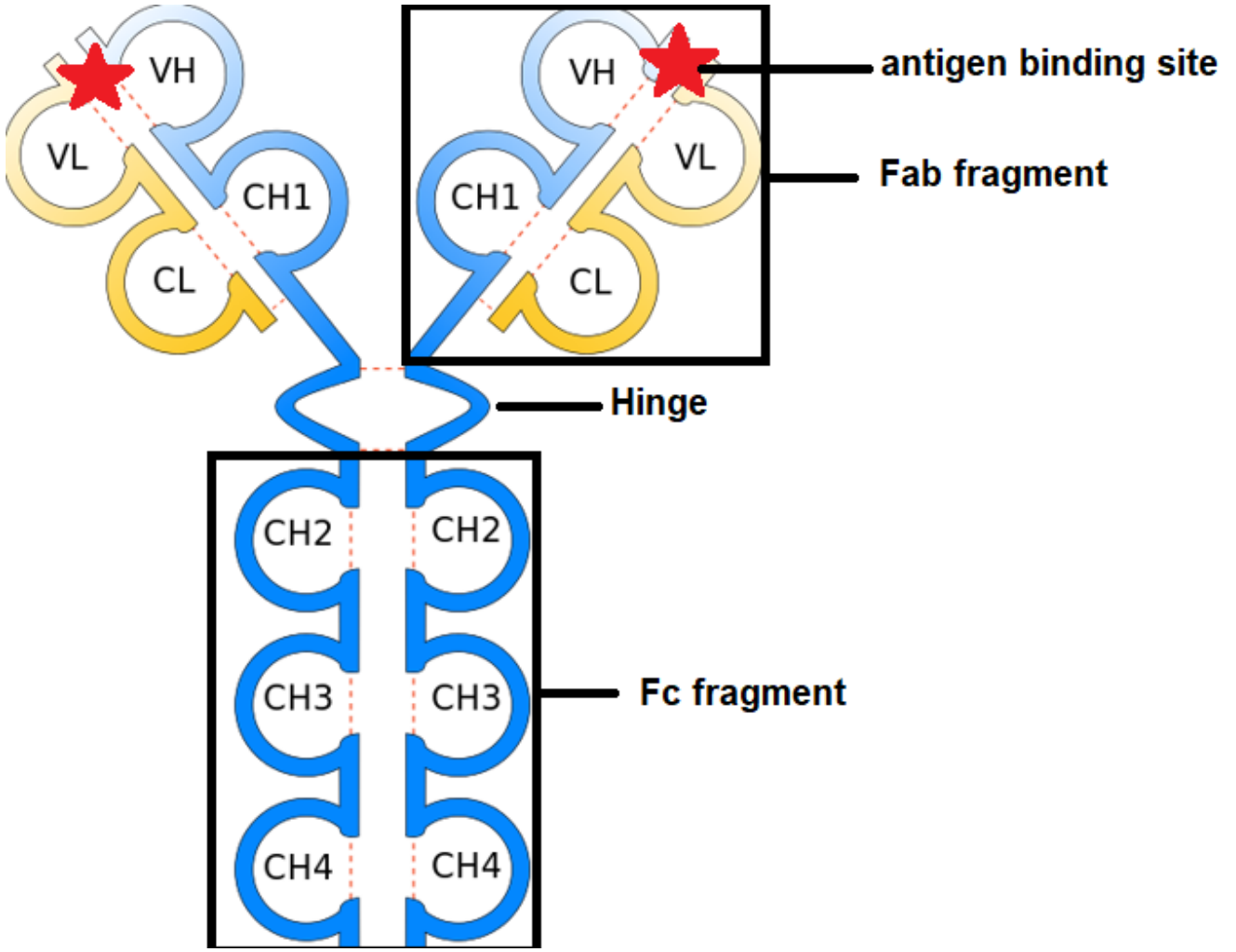
B Cell Activation by an Effector Helper T cell

Costimulation by B7 to CD28 binding

The Adaptive Immune Response: Humoral Immunity 6

- B cell activation includes **clonal expansion and differentiation** of the B cell. Like activated T cells, activated B cells differentiate into both **effector B cells** and **memory B cells**. Memory B cells are less dependent on Helper T cells for their activity.
- Effector B cells are called **plasma cells**. These short-lived cells are **"antibody factories"**.
- As mentioned previously, antibody monomers are Y-shaped protein structures. The two arms of the Y are **Fab regions.** The **stem** of the Y is the **Fc region.**
- Each antibody contains **two heavy chains** (4 constant regions and 1 variable region) **and two light chains** (1 constant region and 1 variable region).
- The **two antigen binding sites** are located at the tips of the Y and are formed from the **variable (V) regions**.

The Adaptive Immune Response: Humoral Immunity 7



Blue=constant regions
Gold=variable regions

The Adaptive Immune Response: Humoral Immunity 8

- **Antibody Classes**
  - The structure of the **stem** of the antibody molecule determines its class. There are **five** antibody classes. Use the mnemonic "MADGE" to remember them.
  - **IgG** is the smallest, most common circulating antibody type (75-80%). It is also present in high amounts in the interstitial fluid. It has the longest half-life of all antibody classes (~3 weeks). It is small enough to cross the placenta.
  - **IgM** is usually present as a pentamer, and is thus the largest antibody type. It represents about 10% of circulating antibodies and tends to remain in the blood due to its size. It is the first antibody type produced by a plasma cell during an immune response. It is the antibody class that most easily activates complement. It has a half-life of 10 days. **IgM monomers** are the most common BCRs.

The Adaptive Immune Response: Humoral Immunity 9

- **IgA** is produced mostly by the plasma cells just deep to the skin and mucous membranes. It exists as a **dimer** and can be transported across epithelial barriers and into body fluids other than blood: saliva, tears, body tract secretions, colostrum and breast milk. It is also known as **"secretory antibody".**
- **IgD** functions as a BCR and is found only in trace amounts (1%) in the blood.
- **IgE** is bound by its stem to the surface of **basophils and mast cells**. Antigen binding by IgE results in degranulation (secretion of histamine and other inflammatory chemicals) by basophils and mast cells. It plays a role in the immune response to helminthes (worms) and is a major force in allergic responses.

The Adaptive Immune Response: Humoral Immunity 10

- **Antibody Class Switching**
  - During the course of a humoral immune response the class of antibody produced by a plasma cell changes. Initially **IgM** is synthesized. Later the plasma cell switches to synthesizing **IgG and still later, possibly IgA, IgD and/or IgE**. Cytokines are required to stimulate class switching.
  - Class switching changes only the **constant (C) regions** of the antibody molecule. The **variable (V) regions** that form the **antigen binding sites** stay the same.
  - All of the antibodies produced by a single plasma cell bind to the **same specific antigen**.

The Adaptive Immune Response: Humoral Immunity 11

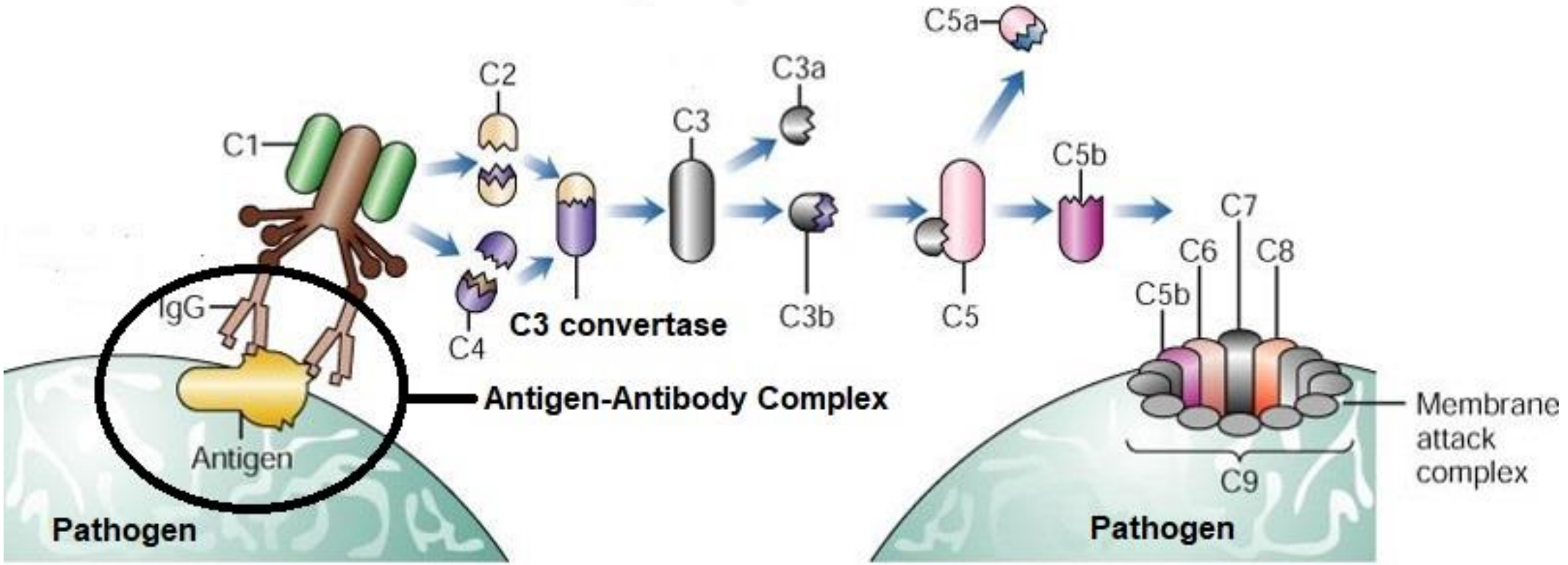
- **Five Antibody Functions**
  - **1. Precipitation**
  - **2. Agglutination**
    - Because antibody molecules have at least two antigen binding sites (IgM pentamer has 10!) they are able to cause antigens to clump together. This makes the antigens more noticeable to phagocytes.
    - If the antigen is **soluble,** the clumping process is called **precipitation**. When antibodies bind to soluble antigens, the antigens become insoluble.
    - If the antigen is cellular (on the surface of a cell), the clumping process is called **agglutination**.
  - **3. Neutralization**
    - Antibodies can inactivate bacterial toxins or virus particles by binding to their component antigen molecules.

The Adaptive Immune Response: Humoral Immunity 12

- **4. Opsonization**
  - When antibodies bind to antigens they opsonize them—making them more noticeable to phagocytes.
  - Macrophages, neutrophils, eosinophils and NK cells have antibody receptors (**Fc receptors**) on their surfaces that bind to the stem end of antibody molecules that are bound to antigen.
- **5. Complement Activation (aka Complement Fixation)**
  - The **classical pathway** of complement activation is triggered by IgM or IgG antibodies bound to antigens on pathogen surfaces. Complement protein C1 binds to the stem of the antibody molecule. Complement activation results in the the release of chemotaxins and inflammatory chemicals as well as formation of MACs.

The Adaptive Immune Response: Humoral Immunity 13

Complement Fixation:



The Immune Response: Passive vs Active

- **Passive immunity**
  - Passive immunity is a means of providing antibodies without activating the recipient's lymphocytes. Protection is immediate. **No memory cells form**.
  - **Natural Passive Immunity**
    - For example, IgG antibodies from a pregnant woman's immune system can **cross the placenta** and enter the body of the fetus she carries. These antibodies are usually helpful. In the case of erythroblastosis fetalis (due to Rh incompatibility) they are harmful.
    - Newborns receive IgA antibodies by ingesting their **mother's breast milk**. The baby's digestive tract is immature and does not break down the antibody protein like an adult's digestive tract would. So the antibodies remain functional and are absorbed across the digest tract wall into the babies bloodstream.

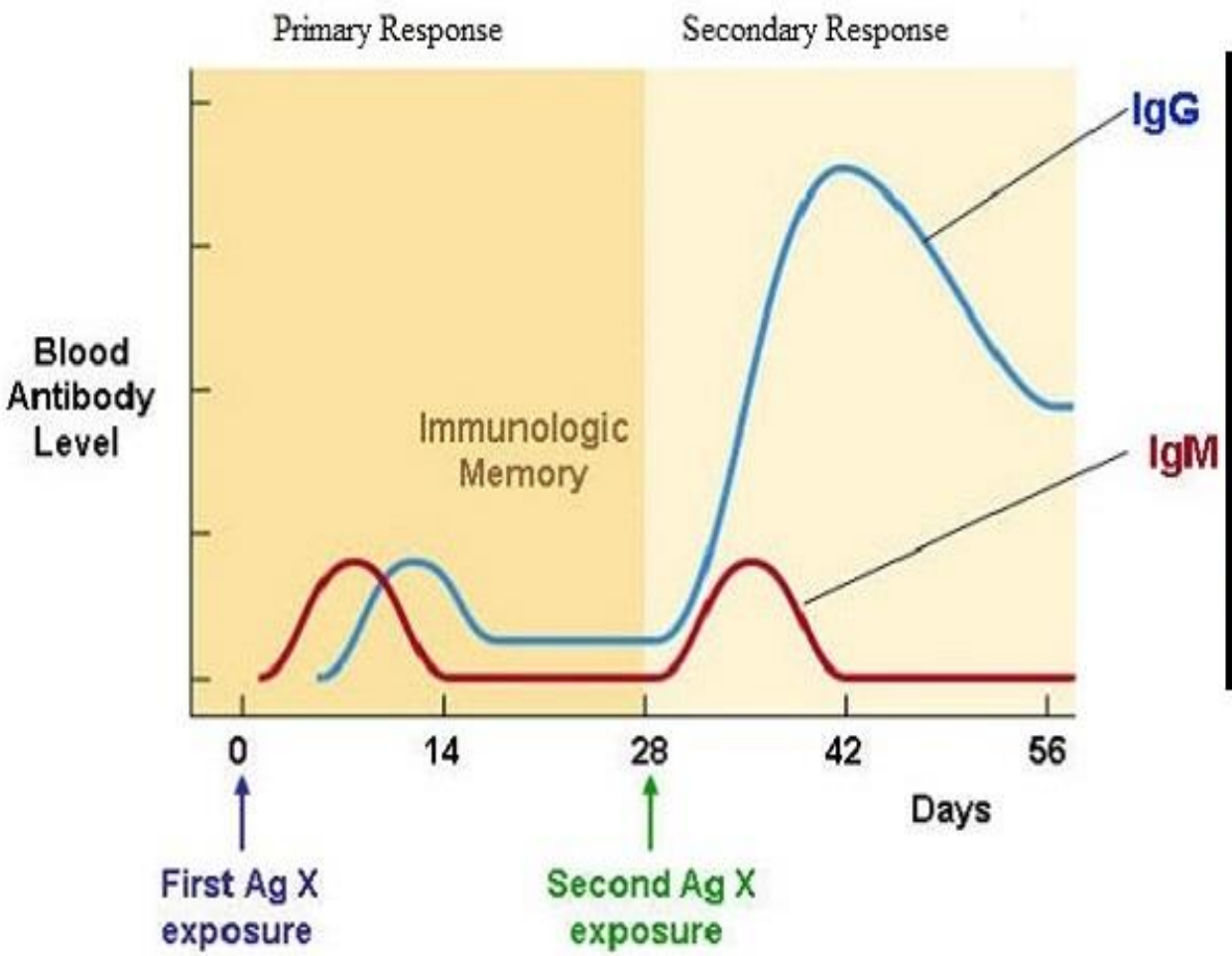
The Immune Response: Passive vs Active 1

- **Artificial Passive Immunity**
  - **Serotherapy** is the direct injection of antibodies (usually IgG directed against toxins) into the body. It is useful when exposure to a serious pathogen (tetanus, botulism, rabies, hepatitis) has occurred or is likely or in the case of certain snake or spider bites. Two examples:
    - If an Rh- woman who is pregnant with an Rh+ fetus is injected with anti-Rh IgA antibodies (**Rho Gam**) any Rh+ fetal cells that enter her circulation will be opsonized, agglutinated and phagocytized thus preventing the activation of the woman's immune system. Erythroblastosis fetalis is thus prevented. IgA is too large to cross the placenta.
    - **Human immune globulin** is a sterile concentrated protein mixture that contains mostly IgG and traces of IgM and IgA from the pooled plasma of several healthy adults. It is used in the treatment of immune system deficiency disorders.

The Immune Response: Passive vs Active 2

- **Active Immunity**
  - **Natural active immunity** has already been described.
  - **Artificial active immunity** mimics the natural primary immune response. It is conferred by a **vaccine**.
  - Vaccines contain dead pathogens or live attenuated (weakened) pathogens, that cannot cause serious disease. **Memory cells** are formed by the primary response and provide a strong, immediate **secondary immune response** upon exposure to live pathogen later in life. The response may be mediated by **memory B cells, memory T cells** or both depending on the vaccine.
    - Vaccination provides the a primary response to an antigen.
    - New Mexico Childhood Immunization Schedule

The Immune Response: Passive vs Active 3



The Secondary Humoral Immune Response

Occurs faster

Produces more antibodies

Produces antibodies with higher affinity

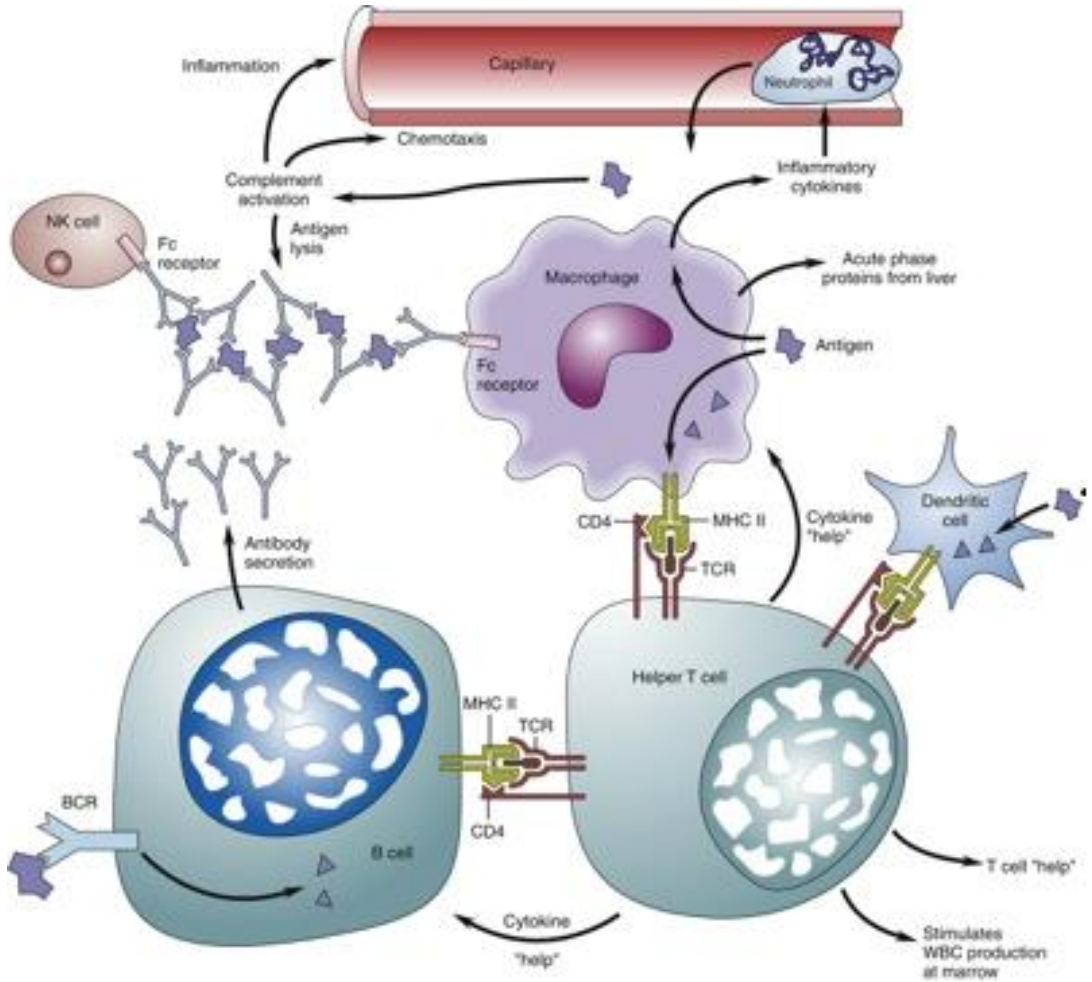
Is mediated by memory cells

The Immune Response: Integration and Regulation

Integrated Response to New Antigen

- **Macrophages** play a major role in **integration and initiation** of the innate and adaptive immune responses.
  - Upon encounter with a new antigen, macrophages release cytokines and chemotaxins to recruit neutrophils and NK cells (major players in the innate response).
  - Macrophages also act as APCs to initiate T lymphocyte responses to cellular antigens.
  - Macrophages have receptors for antibody stems and complement to assist their recognition of opsonized antigens.
- **Helper T cells** also play a major role in the **integration** of innate and adaptive immune responses.
  - The cytokines released by effector helper T cells stimulate the activity of macrophages, NK cells, neutrophils, cytotoxic T cells, B cells and other helper T cells.

The Immune Response: Integration and Regulation 1



Notice how these cell types play a role in BOTH innate immunity and adaptive immunity.

Macrophages

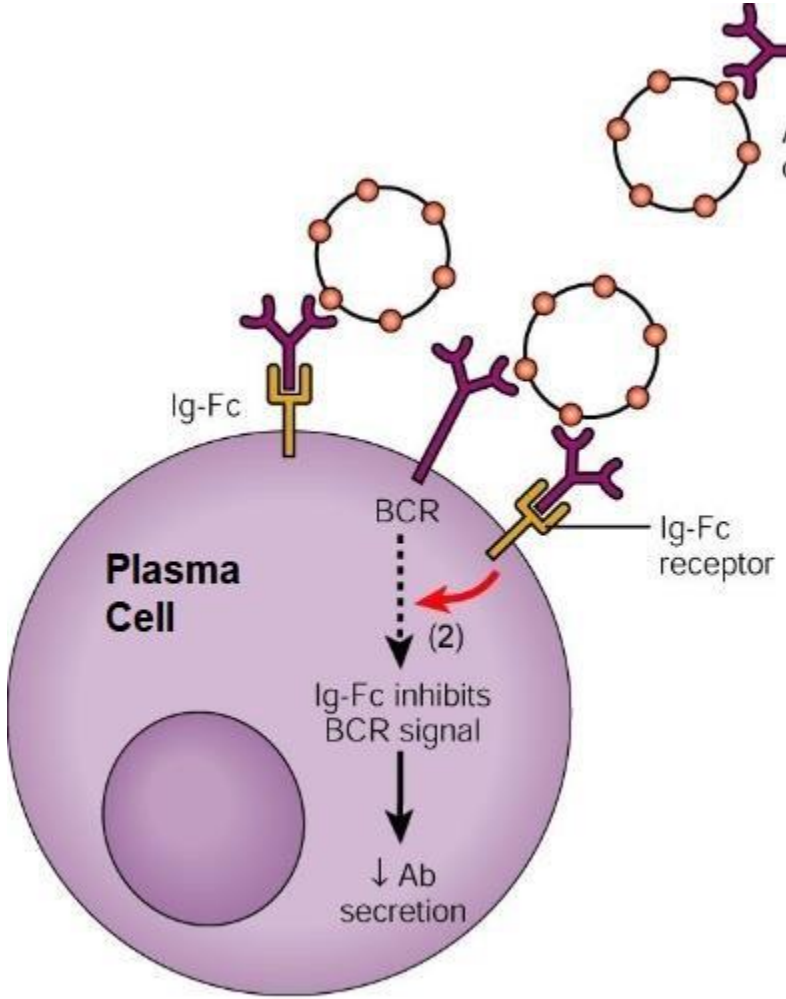
Helper T cells

The Immune Response: Integration and Regulation 2

Immune System Regulation

- Several mechanisms exist to prevent immune responses from causing serious damage to self tissues:
  - Self-tolerance of lymphocytes
  - Antigen dosing controls by APCs
  - Costimulation requirements for lymphocytes
  - Antibodies bind to Fc receptors on plasma cells to exert negative feedback.
  - Suppressor T lymphocytes exist. They secrete cytokines to inhibit immune cells.
  - Eosinophils secrete enzymes that destroy histamine.
  - Inhibitors of inflammatory enzymes and reactive oxygen species exist (anti-proteases, superoxide dismutase, catalase)
  - Corticosteroids, endorphins and enkephalins dampen the immune response.

The Immune Response: Integration and Regulation 3



When antibodies bind to the Fc receptors on a plasma cell that has been synthesizing those same antibodies, the plasma cell will reduce the rate of synthesis of the antibodies.

This is a form of negative feedback.

Alterations in Immune Function

- Alterations in immune function occur as either excessive immune responses or deficient immune responses:
  - **Excessive Immune Responses**
    - Autoimmunity
    - Hypersensitivity
  - **Deficient Immune Responses**
    - Primary Immunodeficiency
    - Secondary Immunodeficiency
    - AIDS

Excessive Immune Responses: Autoimmunity

Autoimmunity occurs when an immune response is directed toward self cells. The process is multifaceted and not well-understood. Multiple theories exist:

- **Antigenic mimicry theory**
  - Infective agents deposit molecules on host cells during infection. Later an immune response is directed against those cells.
  - Guillain-Barre Syndrome often develops following *Campylobacter jejuni* infection.
  - Rheumatic heart disease often develops following streptococcal infection.
- **Sequestered antigen theory**
  - Some self-antigens (cornea of the eye or spermatogenic cells in the seminiferous tubules of the testis) are normally hidden from the immune system throughout life so developing lymphocytes do not develop self tolerance toward them. If exposed due to injury, autoimmunity results.

Excessive Immune Responses: Autoimmunity 1

- **Thymus gland defect theory**
  - Some self-antigens are not presented to T cells during the fetal stage due to a defect in the thymus gland.
  - Generalized autoimmune disorders like serum lupus erythematosus (SLE) may be produced in this way.
- **Defective lymphocyte theory**
  - Suppressor T cells decrease in number or activity
  - Some lymphocytes fail to respond to suppressor T cell cytokines
  - Some defective or self-intolerant B cells produce autoantibodies
- **Genetics theory**
  - Females are at higher risk for autoimmune disorders than males.
  - Abnormally high or low serum levels of certain cytokines occur in autoimmune disorders, reflecting abnormal gene expression.
  - Certain MHC alleles are associated with autoimmune disorders. 95% of persons with **ankylosing spondylitis** have B27 self antigen.

Excessive Immune Responses: Autoimmunity 2

- **Environmental Triggers for Autoimmunity**
  - **Chronic or repeated infection** with certain viruses (Epstein-Barr virus or cytomegalovirus) or bacteria (*Campylobacter jejuni or Heliobacter pylori*) may alter immune cell responses or contribute to antigenic mimicry.
  - **Stress** may provoke the neuroendocrine system to promote the excessive secretion of inflammatory cytokines.
    - Immune cells have been shown to have receptors for neurotransmitters and hormones.
    - Similarly, neurons and endocrine cells have been shown to have receptors for cytokines.
- **Treatment of Autoimmune Diseases**
  - **Immunosuppressive drugs** are used to treat autoimmunity. Unfortunately, these drugs inhibit normal immune responses as well as abnormal immune responses. Side effects are unpleasant and often dangerous due to **risk of infection**.
  - **Plasmapheresis** is dialysis of plasma to remove autoantibodies.

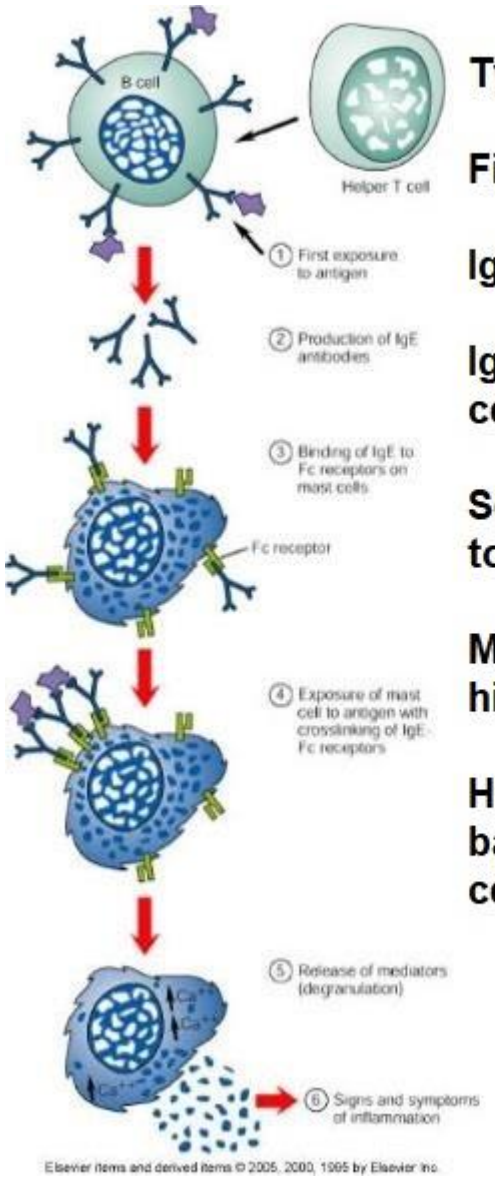
Excessive Immune Responses: Autoimmunity 3

- **Immunosuppressive Medications**
  - **Corticosteroids**
    - Inhibit phospholipases
    - Decrease lymphocyte numbers and activity
    - Ex: prednisone
  - **Cytotoxins**
    - Kill rapidly-dividing cells, immune cells and other cells too
    - Ex: methotrexate
  - **Cyclosporines**
    - Reversibly inhibits helper T cell activity early in the cell cycle
    - Also used in organ transplantation to prevent rejection
  - **Tumor necrosis factor (TNF) inhibitors**
    - Decrease TNF production
    - Inhibits migration of WBCs
    - Ex: etanercept (Embrel), infliximab (Remicade)

Excessive Immune Responses: Type 1 Hypersensitivity

- There are **four** classes (I-IV) of hypersensitivity reactions:
- **Type I Hypersensitivity (aka allergy)**
  - It is a form of **"immediate"** hypersensitivity. A reaction occurs within 10-30 minutes after exposure to an allergen. An allergen is a chemical that is not harmful to the body, but the immune system treats it as a foreign antigen.
  - **Etiology and Pathogenesis**
    - The immune system becomes sensitized to an allergen through **B cell activation** and the production of IgE antibodies by plasma cells. IgE then binds to **mast cells and basophils**. Those cell types have membrane receptors for IgE stems (Fc receptors).
    - Subsequent exposure to the same allergen will cause the allergen to bind to and crosslink IgE on basophils and mast cells. This signals cytoplasmic changes (increased intracellular Ca2+ ions, etc.) that lead to **degranulation**==secretion of histamine.

Excessive Immune Responses: Type 1 Hypersensitivity 2



Type I Hypersensitivity

First exposure: B cell activation

IgE antibodies produced

IgE binds to Fc receptors on mast cells

Second exposure: allergen binds to IgE on mast cells

Mast cell degranulate releasing histamine

Histamine binds to receptors on basophils, mast cells and other cells>>>inflammation!

Excessive Immune Responses: Type 1 Hypersensitivity 3

- Basophils and mast cells are the primary effector cells in allergic reactions but essentially all WBC types have **histamine receptors**, as do endothelial and epithelial cells. There are at least four different types of histamine receptors.
- Histamine receptor binding generally causes activation of various protease enzymes and the kinin and arachidonic acid pathways.
- One type of histamine receptor (H2) causes inhibition of inflammatory responses.
- Substances that can act as allergens: medications, insect stings, foods, pollens, various inorganic chemicals.
- Most cases of **asthma** are classified as allergy.
- There is a strong **genetic component** to type I hypersensitivity. Children of two allergic parents have roughly a 50% chance of being allergic. Children of just one allergic parent have about a 30% chance of being allergic.

Excessive Immune Responses: Type 1 Hypersensitivity 4

- **Clinical Manifestations**
  - Common symptoms include:
    - Vascular changes (redness, heat, swelling, pain)
    - Bronchoconstriction (especially in the case of asthma)
    - Rhinitis (especially if allergen is pollen-hay fever)
    - Skin changes: (eczema)
  - In a small number of highly allergic individuals, a life-threatening reaction known as **anaphylaxis** may occur. It may involve **shock due to extreme vasodilation**. Offending allergens are bee stings, peanuts and seafood.
- **Treatments**
  - **Antihistamines**: Benadryl
  - **β receptor agonists**: epinephrine (EpiPen) for food/insect
  - **Corticosteroids**
  - **NSAIDS**
  - **Anticholinergics (ACH inhibitors):** Blocking ACH inhibits parasympathetic activity to better allow for bronchodilation by sympathetic neurons.

Excessive Immune Responses: Type 1 Hypersensitivity 5

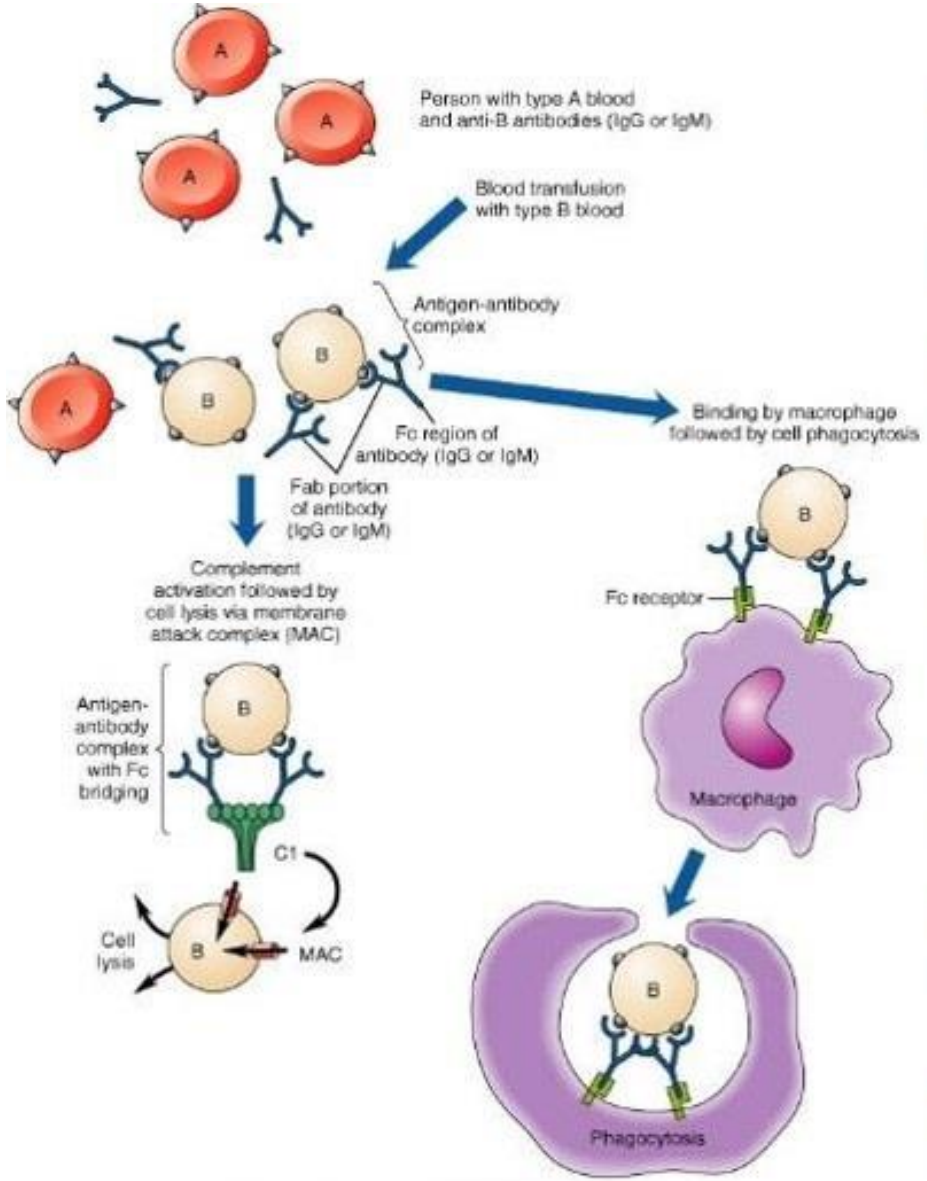
- **Anti-IgG antibodies** (Xolair injection for asthma)
- **Pharmacologic desensitization:** External allergens are strictly avoided, meanwhile allergens are injected regularly over the course of months or years with increasing dose until the patient can tolerate the antigen without reacting.

- **Prevention**
  - During pregnancy the mother should
    - Avoid foods to which she is allergic
    - Eat a variety of foods during the third trimester
    - Avoid whole eggs the last month and while breast feeding
    - Limit cow's milk to two glasses per day
  - During the child's infancy
    - Avoid exposure to environmental pollutants
    - Breastfeed for at least 6 months, supplement with non-cow's milk
    - Feed solids foods only after six months
    - Keep infant's room free of dust, mold, pet dander

Excessive Immune Responses: Type II Hypersensitivity

- **Type II Hypersensitivity (aka cytotoxic hypersensitivity)**
  - The response is usually “immediate”, but in some cases (thyroiditis or myasthenia gravis) it can occur over a longer period of time. It is an antibody-mediated reaction against proteins on the surface of donor human cells or in some cases, self cells. Inflammation can be excessive or absent
  - **Etiology and Pathogenesis**
    - Initial exposure to the antigen activates B cells that become IgG and IgM secreting plasma cells. The antibodies then bind to the antigens on the foreign cells or on self cells.
    - There are three possible results:
      - **Inflammation mediated by complement or Fc receptors**
        - Acute rheumatic fever (streptococcal antigens mimic myosin in cardiac muscle fibers)
        - Transfusion reaction (ABO mismatch) See diagram below.
      - **Cell depletion or destruction without inflammation**
        - Autoimmune hemolytic anemia
        - Autoimmune thrombocytopenia
        - Hemolytic Disease of the Newly Born (Erythroblastosis fetalis)
      - **Cell dysfunction due to antibody binding**
        - Myasthenia gravis; antibodies bind to nicotinic receptors for acetylcholine at the motor end plate of skeletal muscle fibers. Muscle weakness occurs. See diagram below.
        - Graves disease; antibodies bind to thyroid stimulating hormone receptors on thyroid follicle cells. Thyroid hormone level is very low (hypothyroidism).

Excessive Immune Responses: Type II Hypersensitivity 1



Type II Hypersensitivity (Cytotoxic)

Example: Transfusion Reaction

Type A person receives Type B blood.

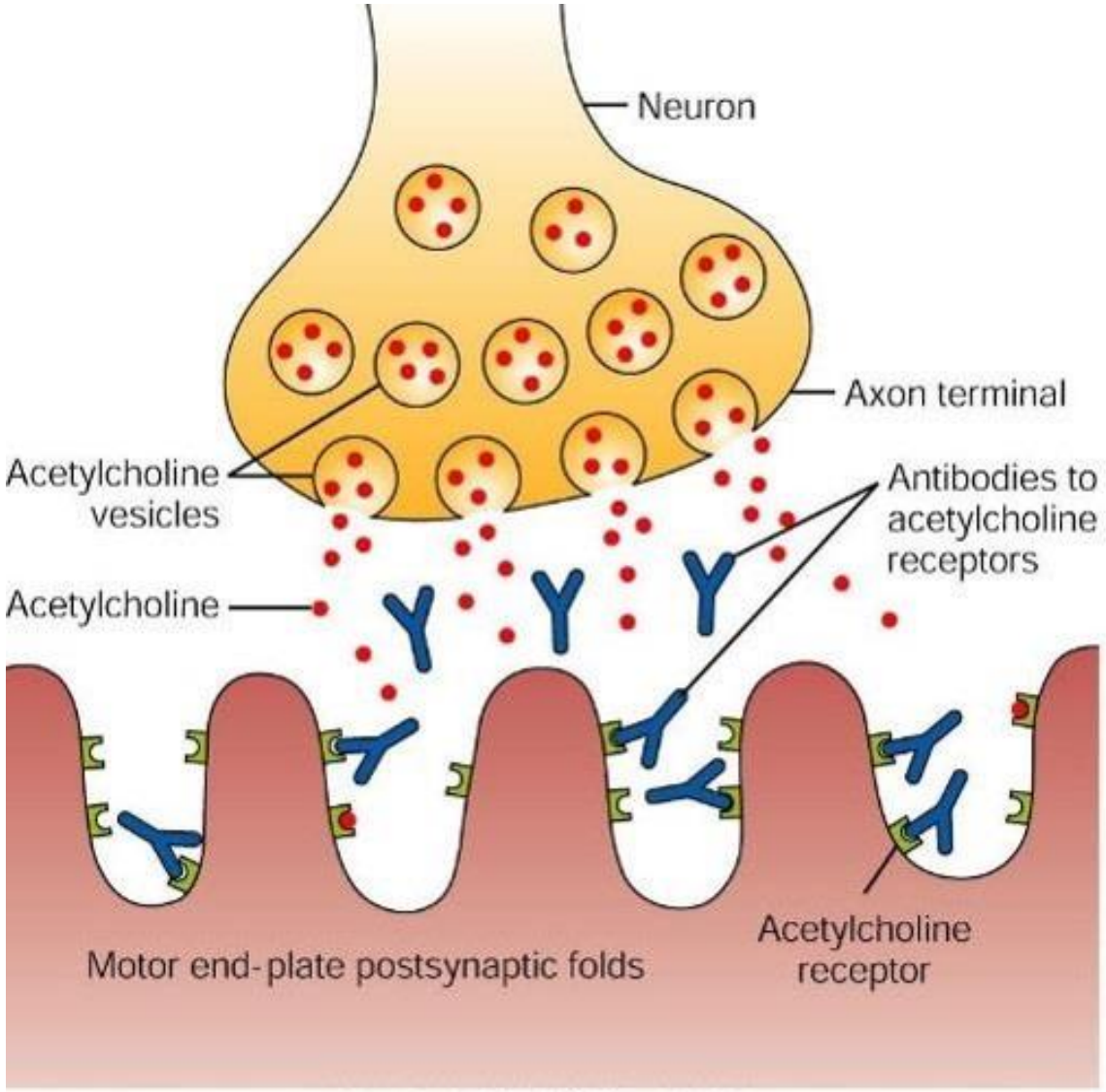
Innate anti-B antibodies bind to Type B red blood cells causing agglutination

Complement fixation by IgG or IgM monomers occurs.

Type B cells are lysed by MAC complexes

Type B cells are opsonized by complement and phagocytized

Excessive Immune Responses: Type II Hypersensitivity 2



Type II Hypersensitivity (Cytotoxic)

Example: Myasthenia Gravis

Autoantibodies bind to nicotinic receptors for the neurotransmitter, acetylcholine (ACH), on skeletal muscle cell membrane

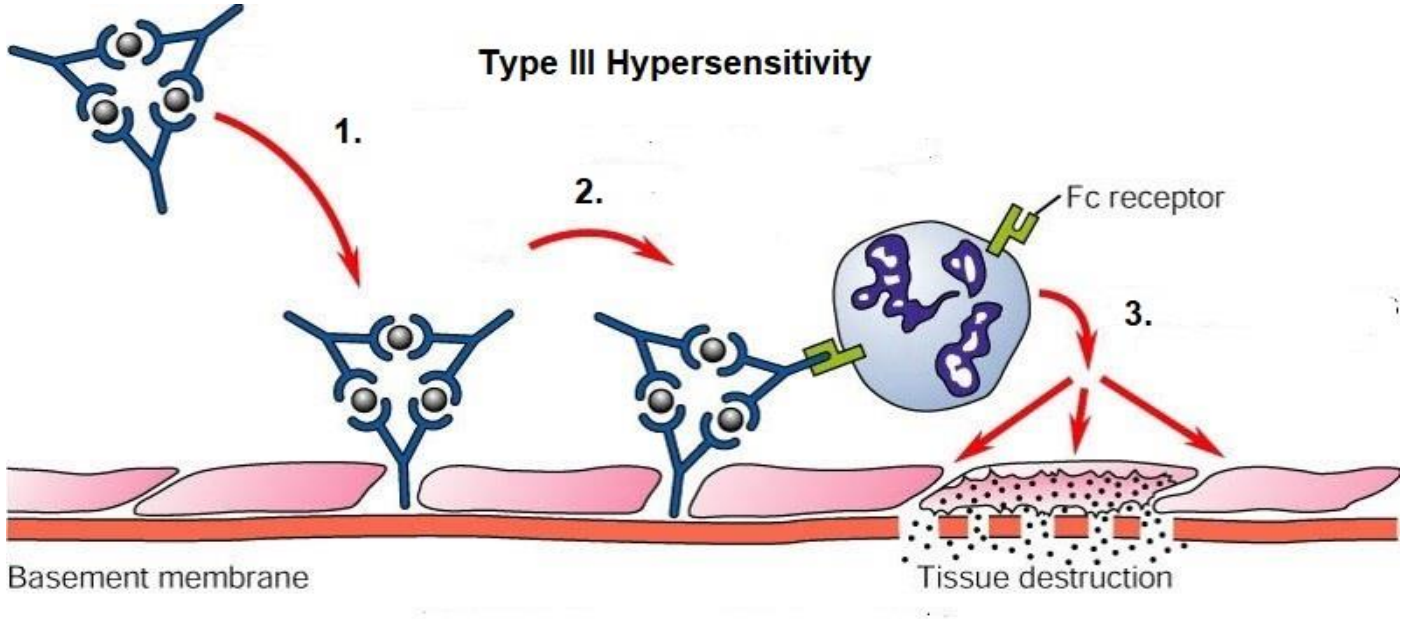
Inflammation does not occur, but skeletal muscle cells suffer disfunction.

Lecture 3D:
Excessive Immune Responses
Deficient Immune Responses
HIV/AIDS

Excessive Immune Responses: Type III Hypersensitivity

- **Type III Hypersensitivity (aka immune complex or Arthus reaction)**
  - Antigen-antibody complexes form, but are not cleared by phagocytes. They become lodged in tissues, complement is activated by the classical pathway and Inflammation is prolonged. The reaction is not immediate. It peaks at about 6 hours post-exposure.
  - **Etiology and Pathogenesis**
    - The reaction is mediated by IgG and IgM, complement, and innate immune cells (neutrophils and mast cells).
    - Antigens fall into three categories: **microbial** molecules, **inhaled** environmental (mold, plant, animal) molecules and **self molecules** that provoke autoantibody formation. Type III antigens are NOT cellular. Type III antigens are precipitated by antibodies and then deposit in tissue.

Excessive Immune Responses: Type III Hypersensitivity 1



1. Antibody-antigen complexes become lodged in tissue (often capillary walls).

2. Fc receptors on immune cells bind to antibody-antigen complexes.

3. Inflammatory chemicals damage tissue and basement membranes.

Excessive Immune Responses: Type III Hypersensitivity 2

- The type III inflammatory response to the antigen-antibody complexes formed from microbial or inhaled antigens causes the **exposure of autoantigens** in damaged tissue. B cells react by forming autoantibodies thus creating even more antigen-antibody complexes.
- Size matters!
  - **Small** antigen-antibody complexes escape the notice of phagocytes and may be cleared by the kidneys.
  - **Large** antigen-antibody complexes are more susceptible to phagocytes but are too large for kidney clearance.
  - **Medium**-size complexes tend to persist. They don't fix complement very well so they aren't as attractive to phagocytes, and they are too large for kidney clearance.

Excessive Immune Responses: Type III Hypersensitivity 3

- **Examples of Type III Hypersensitivities**
  - **Immune complex glomerulonephritis**: Antibodies are formed against streptococcal or staphlococcus bacterial antigens following kidney infection. Complexes become lodged in glomerular capillary basement membranes of kidney nephrons and cause inflammation there.
  - **Clinical Manifestations:** decreased urine formation, protein, blood and RBC casts in the urine. Renal failure may result.
  - **Serum lupus erythematosus (SLE)**: Autoantibodies are formed against host cell nuclear material (DNA and RNA). Complexes become lodged in basement membranes throughout the body.
  - **Clinical Manifestations**: depend on site of complex deposition.
    - Kidneys: glomerulonephritis
    - Skin: butterfly shaped rash across the nose; rash on sun exposed skin areas

Excessive Immune Responses: Type III Hypersensitivity 4

- **Joints:** arthritis
- **Lungs:** pleurisy, restrictive pulmonary disease
- **Heart:** pericarditis
- **Bone marrow:** anemia, thrombocytopenia,
- **Digestive tract:** gastrointestinal ulcers
- **CNS:** neuritis, seizures, depression, psychosis

- A positive **ANA (antinuclear antibody) test** is usually present in SLE.
- **Treatments for SLE**
  - **Antimalarial drugs** (contain quinine derivatives) are the cornerstone of treatment for SLE. The mechanisms of action is interference with TLR (toll-like receptor) recognition of nuclear antigens. A major side effect is ocular toxicity.
  - **Corticosteroids** and **NSAIDS** are also useful.
  - For severe cases, **cytotoxic drugs** are used.

Excessive Immune Responses: Type III Hypersensitivity 5

- **Antibiotics** are often prescribed due to the increased risk associated with immunosuppressive treatments.
- Lupus patients should **avoid the sun** due to increased photosensitivity of their skin.

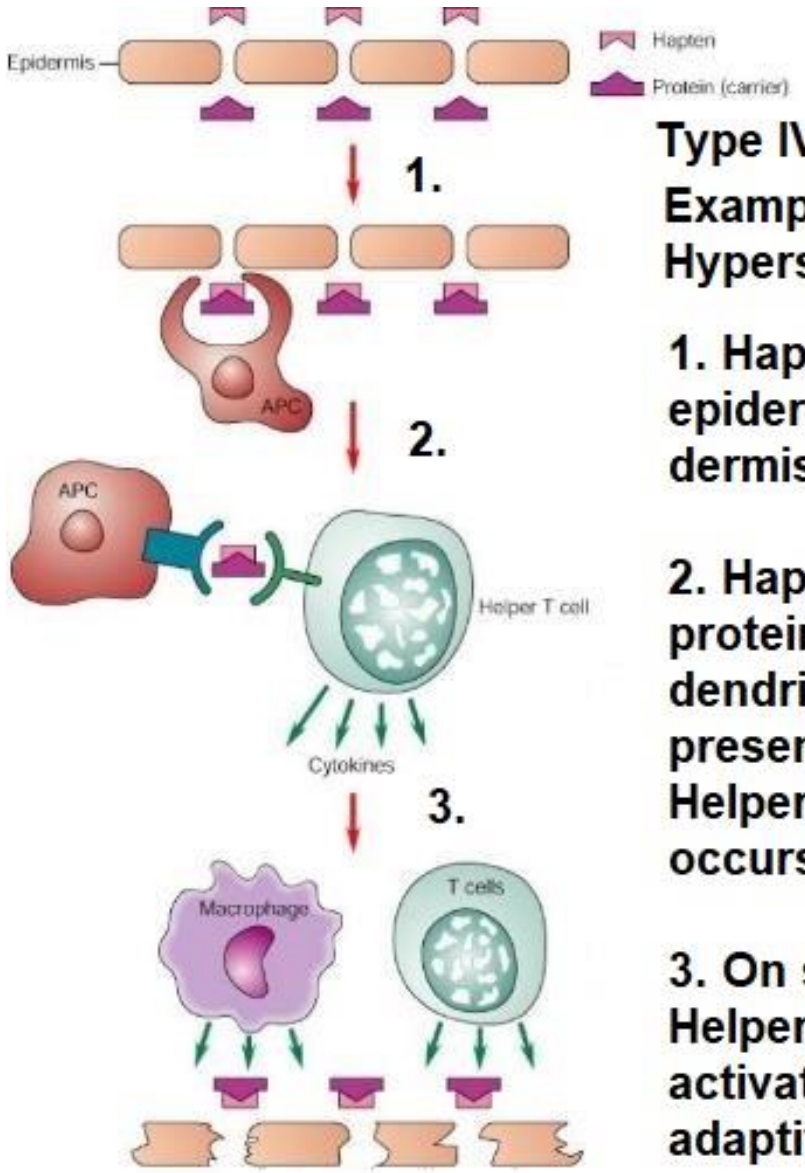
Excessive Immune Responses: Type IV Hypersensitivity

- **Type IV Hypersensitivity**
  - **Etiology and Pathogenesis**
    - **A delayed reaction** occurs and peaks 24 hours to 14 days post-exposure. Early in the reaction **mast cell** degranulation causes recruitment of macrophages and T cells. **Neutrophils, B cells and antibodies are NOT involved**. Symptoms are usually confined to the skin.
  - **Examples of Type IV Hypersensitivities**
    - Contact hypersensitivity
    - Cutaneous basophil hypersensitivity
    - Tuberculin type hypersensitivity
    - Granulomatous type hypersensitivity.

Excessive Immune Responses: Type IV Hypersensitivity 1

- **Contact hypersensitivity** is the most familiar form of type IV reaction. It occurs as a reaction to haptens ("half antigens"). Haptens are **lipid-soluble molecules** and thus can penetrate the epidermis of the skin after being touched. Haptens are associated with various plant oils (poison ivy), cosmetics, latex, elastics, adhesives, ointments, metals (nickel), etc.
- Haptens become antigenic in the dermis of the skin by binding to a self protein. Resident **dendritic cells (Langerhans cells)** and interpret the hapten/self protein as a foreign antigen. Dendritic cells migrate to lymph nodes and present the "antigen" to activate helper T cells. Upon subsequent exposure to antigen memory helper T cells mediate a response
- The result is a red, blistery, itchy rash! The reaction begins to subside at about 72 hours.

Excessive Immune Responses: Type IV Hypersensitivity 2



Type IV Hypersensitivity

Example: Contact Hypersensitvity

1. Hapten penetrates the epidermis and enters the dermis.

2. Hapten binds to self protein and is engulfed by a dendritic cell. Dendritic cell presents antigen to a naive Helper T cell. Sensitization occurs.

3. On second exposure, Helper T cell cytokines activate both innate and adaptive responses.

Excessive Immune Responses: Type IV Hypersensitivity 3

- **Cutaneous basophil hypersensitivity** occurs in the immune rejection of skin grafts. It is the fastest form of type IV hypersensitivity, peaking at 24 hours after exposure. It is mediated by **Helper T cells** that release cytokines to recruit **basophils.** The reaction usually lasts 7-10 days.
- **Tuberculin-type hypersensitivity** is the basis for the **Mantoux skin test for tuberculosis**. If a person previously exposed to TB receives a dermal injection of TB antigen, a skin reaction similar to a contact hypersensitivity response occurs. The amount of antigen injected is very small so the reaction is usually mild and short-lived.
- **Granulomatous hypersensitivity** is a chronic type IV hypersensitivity mediated by lymphocytes and macrophages against antigens that cannot be cleared by phagocytes.

Excessive Immune Responses: Type IV Hypersensitivity 4

- **Phagocytosis fails for one of two reasons:**
  - Phagolysosome formation does not occur, as in **tuberculosis and leprosy (both due to intracellular *Mycobacteria* infections).**
  - The antigen survives the lysosomal enzymes of the phagocyte, as in reactions to **talc and certain suture materials.**
- Persistence of the antigen leads to **granuloma** formation as previously described for chronic inflammation.
- Death of granulomatous tissue is termed **caseous necrosis**.
- Activated fibroblasts within the granuloma lay down increasing amounts of fibrous tissue causing **scar formation**.

ACID

- A mnemonic device for hypersensitivity reaction types, ACID.
- A-type 1 is **a**llergy
- C-type 2 is **c**ytotoxic
- I-type 3 is **i**mmune complex
- D-type 4 is **d**elayed

Deficient Immune Responses

- **Deficient Immune Responses: Primary Immunodeficiency**
  - **Primary immunodeficiencies** are attributable to immune system disorders. They are usually inherited, but may be acquired. Persistent, severe infections are the primary symptoms. They may affect B cells only, T cells only or both cell types. Examples
    - **Disorders that affect both T cells and B cells**: SCIDs, Wiskott-Aldrich Syndrome
    - **T cell disorders:** DiGeorge Syndrome, Chronic Mucocutaneous Candidiasis
    - **B cell disorders:** IgA Deficiency, Bruton X-linked Agammaglobulinemia

Deficient Immune Responses: Primary Disorders Affecting T Cells and B Cells

- **SCIDs (Severe Combined Immunodeficiencies)** are inherited primary disorders characterized by severely reduced number of T cells. B cells and NK cells may also be affected.
- Recall that when a TCR on a naïve CD4 T cell binds with its antigen as presented by an APC, the CD4 T cell is triggered to synthesize both IL2 and IL2R. IL-2 binding to IL-2 receptor then triggers activation of enzymes that catalyze the reactions required for full activation of the CD4 T cell.
- There are four major types of classic SCID based on the affected cell type(s).

Deficient Immune Responses: Primary Disorders Affecting T Cells and B Cells 1

- **T-B+NK- SCID** affects both T cells and NK cells. It is caused by one of two mutations that affect the **receptor for IL-2**.
  - The **X-linked form** is due to mutation in **IL-2RG**, the gene that codes for the gamma subunit of the IL-2 receptor. It is much more common in males than in females.
  - The **autosomal form** is due to mutation in the **JAK3** gene. That gene codes for Janus tyrosine kinase, a type of **protein kinase** enzyme associated with activation of the IL-2R (IL2 receptor). It is required for signal transduction from IL-2R through the plasma membrane of the CD4 cell.
- **T-B-NK+ SCID** affects T cells and B cells. It is caused by mutation in one of two autosomal genes, **RAG1 or RAG2**. The RAG genes code for **recombinase** enzymes that jumble the genetic sequences of the genes that code for the huge array of antibody molecules, BCRs and TCRs. If the RAG genes are not functioning the body is unable to respond to many antigens.

Deficient Immune Responses: Primary Disorders Affecting T Cells and B Cells 2

- **T-B-NK- SCID** affects all three cell types. It is due to mutation in one of two genes that code for enzymes involved in lymphocyte function.
  - The autosomal **ADA** gene codes for **adenosine deaminase**, an enzyme required to convert naïve CD4 cells (Helper T cells) to effector CD4 cells.
  - The autosomal **AD2** gene codes for **adenylate kinase**, an enzyme required for development of bone marrow cells. Mutation in AD2 causes the **most severe form of SCID**, **reticular dysgenesis**. It not only affects T, B and NK cells, but also depresses neutrophil counts and is associated with hearing defects.
- **T-B+NK+ SCID** affects only T cells. It is due to mutation in the autosomal gene for the **IL7R**, interleukin 7 receptor. IL7 is involved in T cell activation, but doesn't affect B or NK cells.

Deficient Immune Responses: Primary Disorders Affecting T Cells and B Cells 3

- **Clinical Manifestations and Treatment of SCID**
  - Infants born with SCID are ill by the age of 3 months. They are extremely susceptible to opportunistic infections, most commonly: *Candida albicans, Pneumocystis carinii*, cytomegalovirus (CMV), herpes virus, varicella (chicken pox) virus and measles virus. Infections are medical emergencies due to the risk of sepsis. Protective isolation of SCID children is advisable.
  - **Curative** treatment is hematopoietic stem cell transplant with cord blood or bone marrow, usually from a tissue matched sibling. Other treatments: long term antibody therapy, enzyme replacement therapy and gene therapy. Before the existence of curative treatment SCID patients had to live life in protective isolation as “bubble” boys and girls.

Deficient Immune Responses: Primary Disorders Affecting T Cells and B Cells 4

SCID (X-linked T-B+NK-) patient, David Vetter, lived in protective isolation his entire life. He died at age 12 in 1983. See more

Deficient Immune Responses: Primary Disorders Affecting T Cells and B Cells 5

- **Wiskott-Aldrich Syndrome (WAS)** is an X-linked recessive disorder affecting both T cell and B cell activity. The mutation is in the WAS gene that codes for a cytoplasmic signaling protein required for cytoskeleton organization in lymphocytes. The result is:
  - Abnormal antibody production and antibody type switching in plasma cells. IgM levels are low and the levels of the other antibody types are either high or low.
  - T cells are present but have low activity.
  - Platelets are deficient=thrombocytopenia, but the relationship to the WAS gene is unknown.

Deficient Immune Responses: Primary Disorders Affecting T Cells and B Cells 6

- **Clinical Manifestations and Treatment of WAS**
  - **Symptoms include:**
    - Eczema (dermatitis)
    - Purpura (flat red skin patches due to dermal bleeding)
    - Infections: pneumonia, meningitis, otitis media, sepsis
    - Renal disease, malignancy, systemic autoimmunity
  - **Average age of death in untreated children is 3.5 years.**
  - **Treatments include:**
    - Hematopoietic stem cell transplantation is a cure.
    - Antibody therapy
    - Antibiotic therapy
    - Gene therapy

Deficient Immune Responses
Primary T Cell Disorders

- **DiGeorge Syndrome (Thymic Hypoplasia)** is a T cell disorder associated the deletion of part of the long arm of chromosome 22. The genetic defect results in a small or absent thymus gland and therefore a deficiency and abnormality of T cells. B cells are normal in number. The development of other organs is affected too.
- **Clinical Manifestations and Treatment of DiGeorge Syndrome:**
  - Up to 40% of patients have recurrent infections and about 1% have symptoms similar to SCID, depending of the degree of thymus gland function.
  - Other problems: developmental delay, cardiac anomalies, hypoparathyroidism with hypocalcemia, hypothyroidism, esophageal atresia, urogenital anomalies, abnormal facial features (low set ears and mandibular hypoplasia)
  - Thymus gland transplant may be necessary as a treatment for deficient immune function.

Deficient Immune Responses
Primary T Cell Disorders 1

- **Chronic Mucocutaneous Candidiasis** is a T cell disorder caused by one of two genetic etiologies: an autosomal recessive mutation in the gene for IL-17 receptor causing loss of receptivity to IL-17A and IL-17F, OR an autosomal dominant mutation in the gene for IL-17F. As a result T cells are unable to respond to the yeast, *Candida albicans.*
- **Clinical Manifestations and Treatment of CMC:**
  - Severe skin and mucous membrane yeast infections occur. Skin infections cause disfigurement.
  - Antifungal medications are used to moderate infection. There is no known cure.

Deficient Immune Responses
Primary T Cell Disorders 2

DiGeorge Syndrome

Chronic Mucocutaneous Candidiasis

Deficient Immune Responses
Primary B Cell Disorders

- **Selective IgA Deficiency** is the most common primary B cell disorder due to a recessive or dominant autosomal mutation that interferes with the formation of **IgA (secretory antibody)** by plasma cells. Serum IgA and IgA in secretions is greatly reduced. B cells are normal in number but fail to respond to certain interleukins.
- **Clinical Manifestations and Treatment of Selective IgA Deficiency**
  - Infections of body tracts: respiratory, gastrointestinal, urogenital.
  - Production of autoantibodies (including anti-IgA) with high incidence of vascular, endocrine and collagen autoimmune diseases.
  - Exogenous IgA treatment is contraindicated due to the presence of anti-IgA autoantibodies.
  - Infection prevention and management are primary treatments.

Deficient Immune Responses
Primary B Cell Disorders 2

- **Bruton X-linked Agammaglobulinemia (XLA)** is a primary B cell disorder due to a mutation in the *btk* gene on the X chromosome. The **btk (Bruton tyrosine kinase) gene** codes for an enzyme that acts in cytoplasmic signaling. The result is deficient production of B cells by the bone marrow. Serum IgG is very low with no detectable IgM or IgA. Helper T cells are deficient due to lack of stimulation by B cells. Thymic function is normal. Lymphoid organs are poorly developed (tonsils, spleen, lymph nodes, Peyer's patches).
- **Clinical Manifestations of XLA**
  - Recurrent infections most often by *Streptococcus pneumoniae and Haemophilus influenza*: pneumonia, meningitis, otitis media, sinusitis, sepsis.
  - Adults with XLA experience high risk of lung disease and arthritis.

Deficient Immune Responses
Secondary Immunodeficiency

- Treatments for XLA include monthly infusion of antibodies and prevention of infection by antibiotic administration. Passive immunotherapy is not always successful causing death in many children before the age of 6. XLA children should not be immunized with live virus vaccines. Gene therapy, if successful, is curative.

- **Secondary Immunodeficiency** may be due to physical, psychosocial, nutritional, environmental or pharmacologic factors.
  - Excessive **neuroendocrine response to stress** (corticosteroid release) increases the risk of infection, but decreases the risk of autoimmunity. Defective neuroendocrine response to stress decreases the risk of infection, but increases the risk of autoimmunity.
  - **Major surgery** depresses the bone marrow for about a month.

Deficient Immune Responses
Secondary Immunodeficiency 1

- **Removal of the spleen** reduces the response to encapsulated bacteria (*S. pneumoniae, H. influenza, S. aureus*).
- **Diseases** such as diabetes, cirrhosis, severe burns, severe trauma, sickle cell anemia, malignancies and severe infections can cause secondary immunodeficiency.
- **Cancer chemotherapy and radiation therapy** kill rapidly dividing cells (bone marrow cells), so immune responses become deficient.
- **Drugs:** anesthetics, alcohol, antibiotics, antithyroids, anticonvulsants, antihistamines and steroids have been linked to secondary immunodeficiency.
- **Malnutrition:** protein and calorie depletion, vitamin deficiency, mineral deficiency (zinc)
- **Over-nutrition:** hyperlipidemia

Deficient Immune Responses
Secondary Immunodeficiency 2

- **Advanced age:** The response (both speed and extent) to antigen challenge decreases in the elderly. Repair mechanisms take longer and may not be completely effective.

- NOTE: **Pregnancy** represents a **proinflammatory** state. Many factors are released from the placenta into the maternal circulation. Failure of the mother's immune system to modulate the response to such factors can lead to miscarriage or **preeclampsia** (maternal high blood pressure after the 20th week of pregnancy) or **eclampsia** (seizures due due maternal high blood pressure).

HIV Infection and AIDS

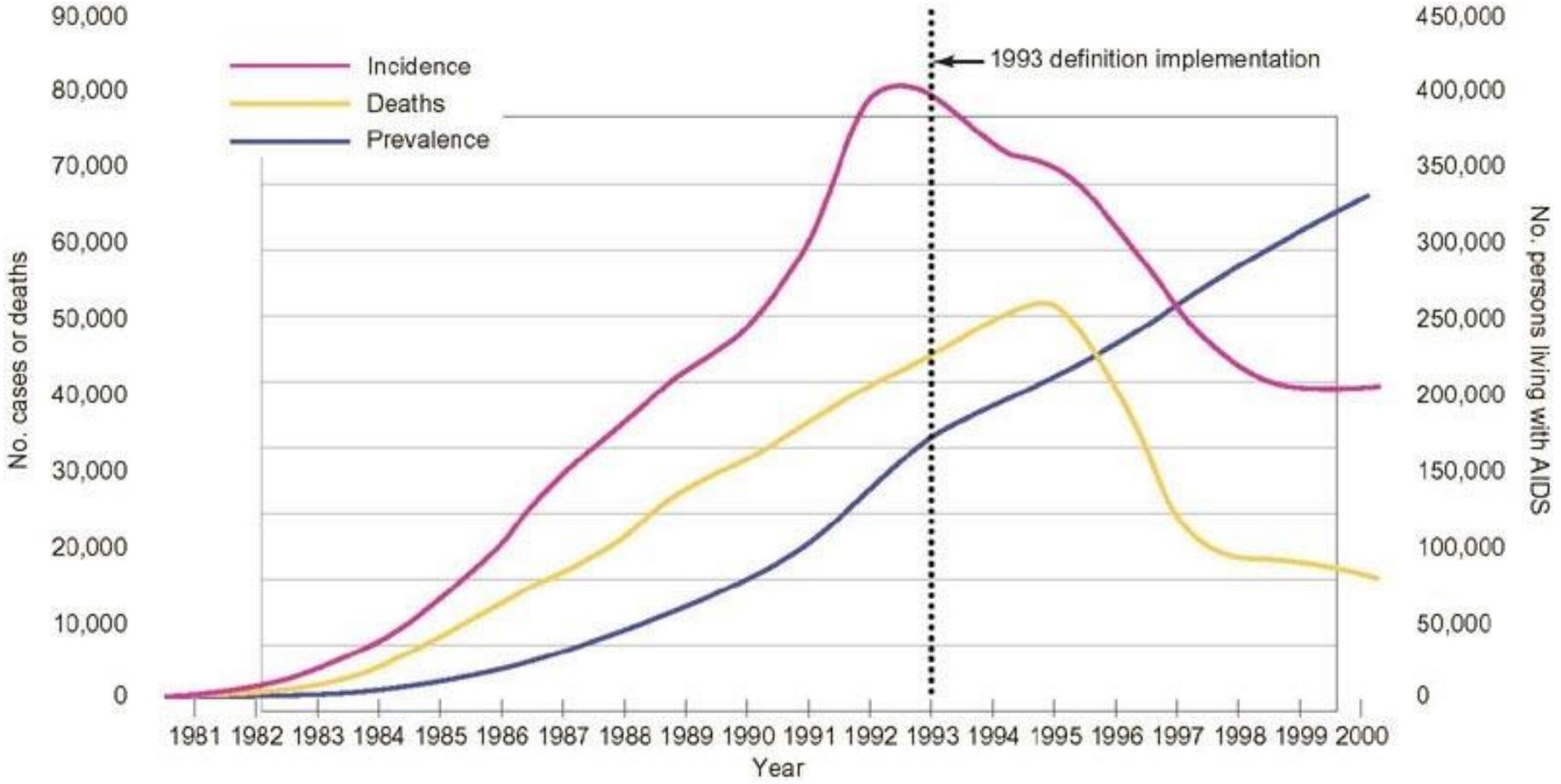
- **HIV Disease** is a primary immunodeficiency caused by viral infection of CD4+ cells. It causes long term severe disease in industrialized countries where preventative care and treatments are available. In third world countries the prognosis for HIV Disease is poor and death rates are high.
- **HIV (Human Immunodeficiency Virus) types 1 and type 2 are retroviruses** that infect mainly Helper T cells, and macrophages to a lesser degree. HIV-1 is the primary agent of infection in the US, Europe and Australia.
- HIV is acquired primarily through **sexual transmission** via semen and vaginal or cervical secretions, **parenteral transmission** via blood by transfusion of blood or blood products or sticks with contaminated needles. **Perinatal transmission** from **mother to child** during pregnancy, during birth, or after birth (through breast milk) also occurs.
- HIV is present in other body fluids (urine, tears, saliva, CSF, amniotic fluid, feces and aerosols) but it is not believed to be transmissible through those fluids.

HIV Infection and AIDS 1

- **High risk groups in the US** include homosexual and bisexual men, intravenous drug users who share needles or syringes, sexual partners of those at high risk and infants born to infected mothers.
- Health care workers are at increased risk due to possibility of needle sticks while treating HIV infected patients.
- In the US incidence of HIV infection and deaths due to HIV infection **peaked in the 1990s**. Since then AIDS prevention and treatment strategies have reduced incidence and deaths considerably, while prevalence has climbed steadily.
- In 2018 1.2 million people in the US were infected with HIV.
- **AIDS has killed more than 650,000 Americans** since it was first reported in the US in 1981. In 1992 AIDS took about 400,000 lives. In 2010 that number was down to about 15,500. But in 2018 that number rose to 15,820 deaths.
- In 2018, 34.9 million people worldwide were living with HIV. Worldwide, **Sub-Saharan Africa** is the site of 66% of all new cases of infection. Treatment is available there, but most people cannot afford it. Infections occur mostly through heterosexual contact and from mother to child.

HIV Infection and AIDS 2

AIDS Incidence, Deaths, and Prevalence in the US 1981-2002

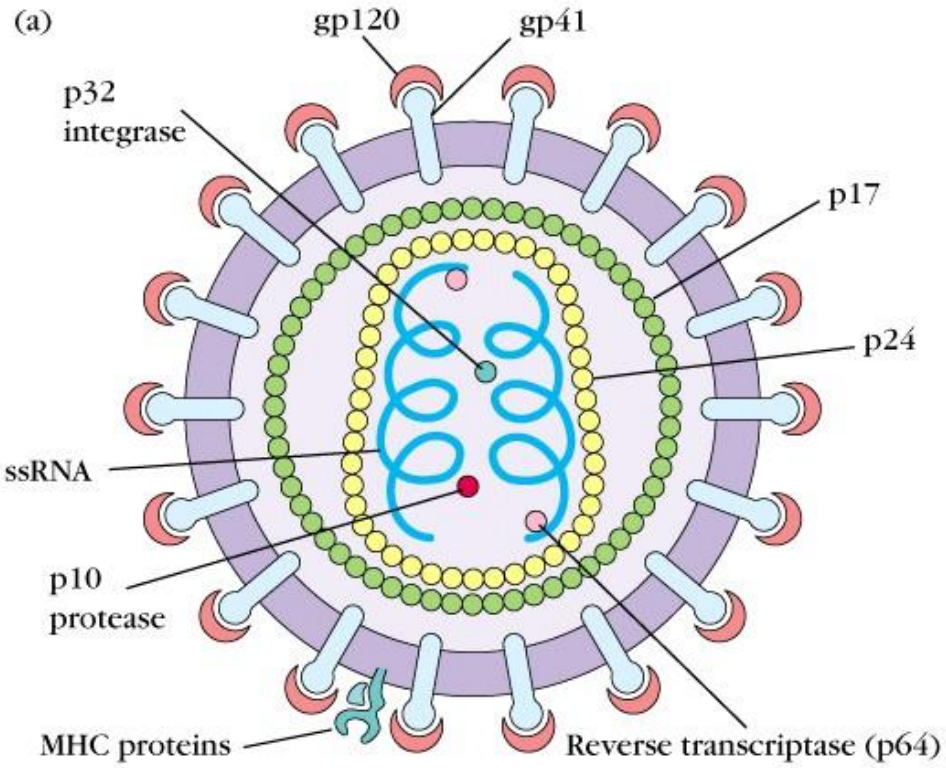


From Update: AIDS-United States, 2000, MMWR Morb Mortal Wkly Rep 51[27]:592-595, 2002.

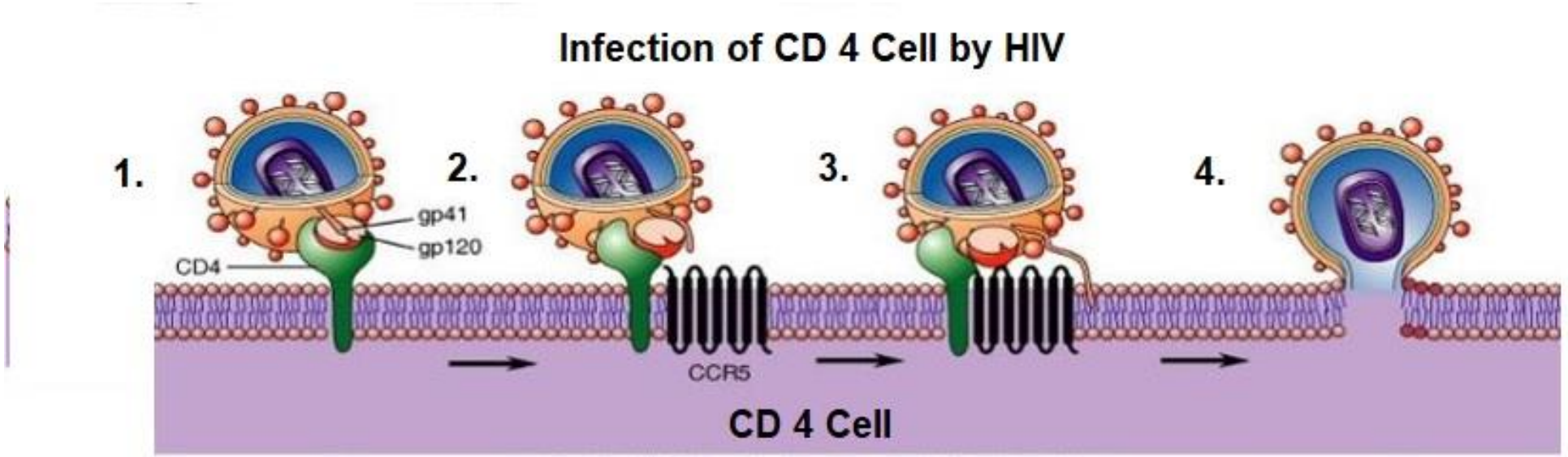
HIV Infection and AIDS: Etiology

HIV Structure (100 nm diameter)

- **Nucleocapsid (Virus Core)**
  - 2 strands of +RNA
    - 9 genes to make new viral proteins
      - pol gene-Reverse transcriptase
      - env gene-viral envelope proteins gp 120 and gp 41
      - viral protease gene
      - regulatory genes
  - Viral proteins from previously infected cells
    - reverse transcriptase, integrase, protease
  - The RNA and protein are surrounded by a capsid layer constructed of protein p24
- **Matrix**
  - Layer of p17 protein between the core and the envelope.
- **Viral Envelope**
  - Lipid bilayer derived from host cell membrane
  - Envelope proteins
    - gp120
    - gp41



HIV Infection and AIDS: Etiology 1



1. gp120 viral envelope protein binds to CD 4 protein on the WBC membrane.

2. gp120 changes shape and binds to chemokine receptor (CCR5) on the WBC membrane.

3. gp41 viral envelope protein implants itself into the CD 4 cell membrane.

4. Viral envelope and CD 4 cell membrane fuse, and the virus core enters the cell.

HIV Infection and AIDS: Etiology 2

1. Reverse transcriptase converts viral +RNA into viral DNA.
2. Viral DNA enters the nucleus.
3. Integrase splices the viral DNA into the host cell's DNA. Viral DNA replicates along with host cell DNA.
4. Viral DNA is transcribed into new viral +RNA molecules.
5. Some viral RNAs are translated into viral proteins; others become part of new virus cores.
6. Viral protease cleaves new viral proteins into smaller sizes.
7. New virus particles are assembled, each including a piece of host cell plasma membrane as its envelope.
8. New virus particles bud from the surface of the host cell.

The HIV Life Cycle

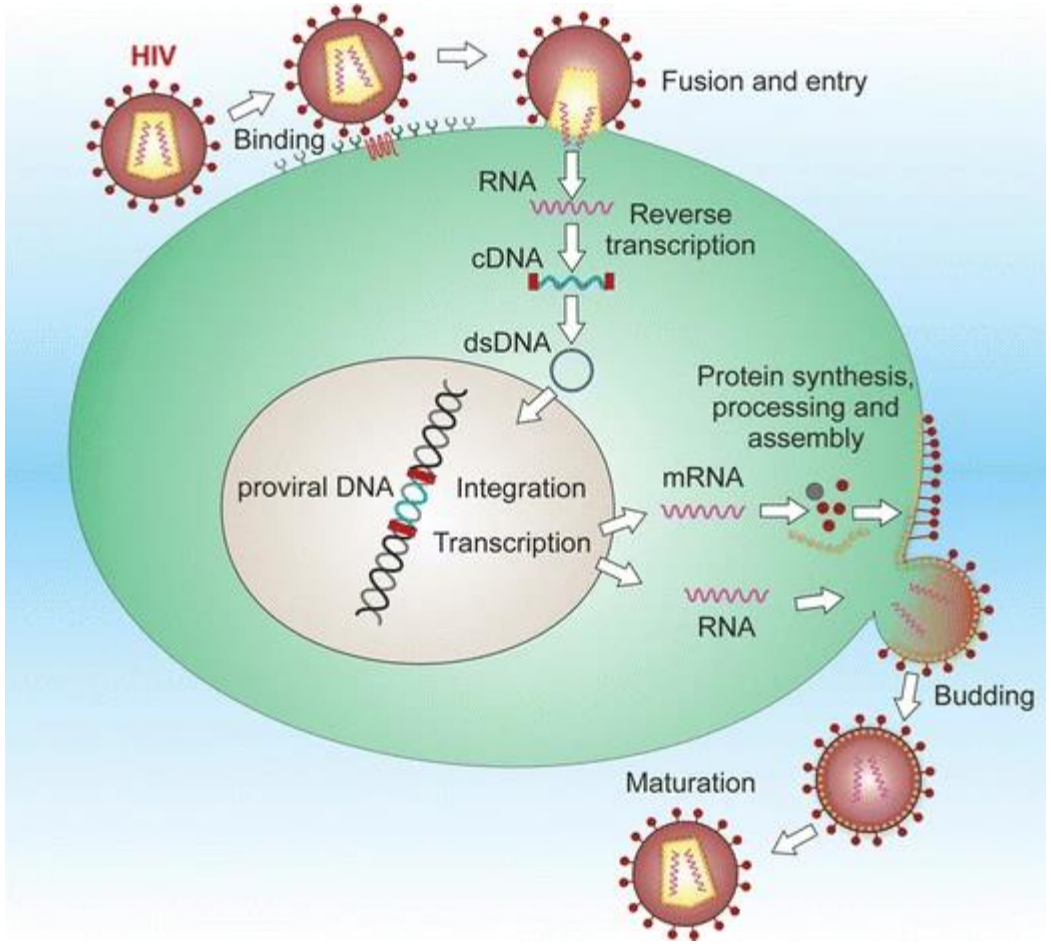


Image: Giovanna De Chiara, Wikimedia Commons

HIV Infection and AIDS: Pathogenesis

- **Effects of HIV Infection on Host Immune Cells**
  - **CD4+ cells are sick and dying.**
    - Living but infected T cells are inappropriately activated.
    - Living but infected macrophages display defective phagocytosis, chemotaxis and antigen presentation.
  - **B cells are normal in number but display abnormal behavior.**
    - Overproduction of nonessential antibodies.
    - Production of autoantibodies against blood cells, platelets, nuclear proteins, sperm, etc.
    - Anti-HIV antibodies are produced, but they are not effective.
  - **Envelope glycoproteins (gp120 and gp41) are the reason for the success of HIV infection.**

HIV Infection and AIDS: Pathogenesis 1

- **How HIV infection Kills CD4+ Cells**
  - Infection produces **huge numbers of progeny viruses**. The gastrointestinal tract is the major site of HIV reproduction (inside lamina propria CD4+ cells).
  - gp120 and gp41 are coated with carbohydrates (glycosylated). This makes HIV appear as "self" to some immune cells.
  - CD4+ cell death may occur by several routes:
    - Loss of cell membrane due to viral budding.
    - Loss of normal host cell protein synthesis
    - Attack by cytotoxic T cells or NK cells
    - Effects of HIV gp120 (**most common cause of death**)
      - ◆gp120 is shed from the virus surface and induces massive anti-gp120 synthesis. gp120 then binds to CD4 on uninfected cells; anti-gp antibodies bind to the gp 120.

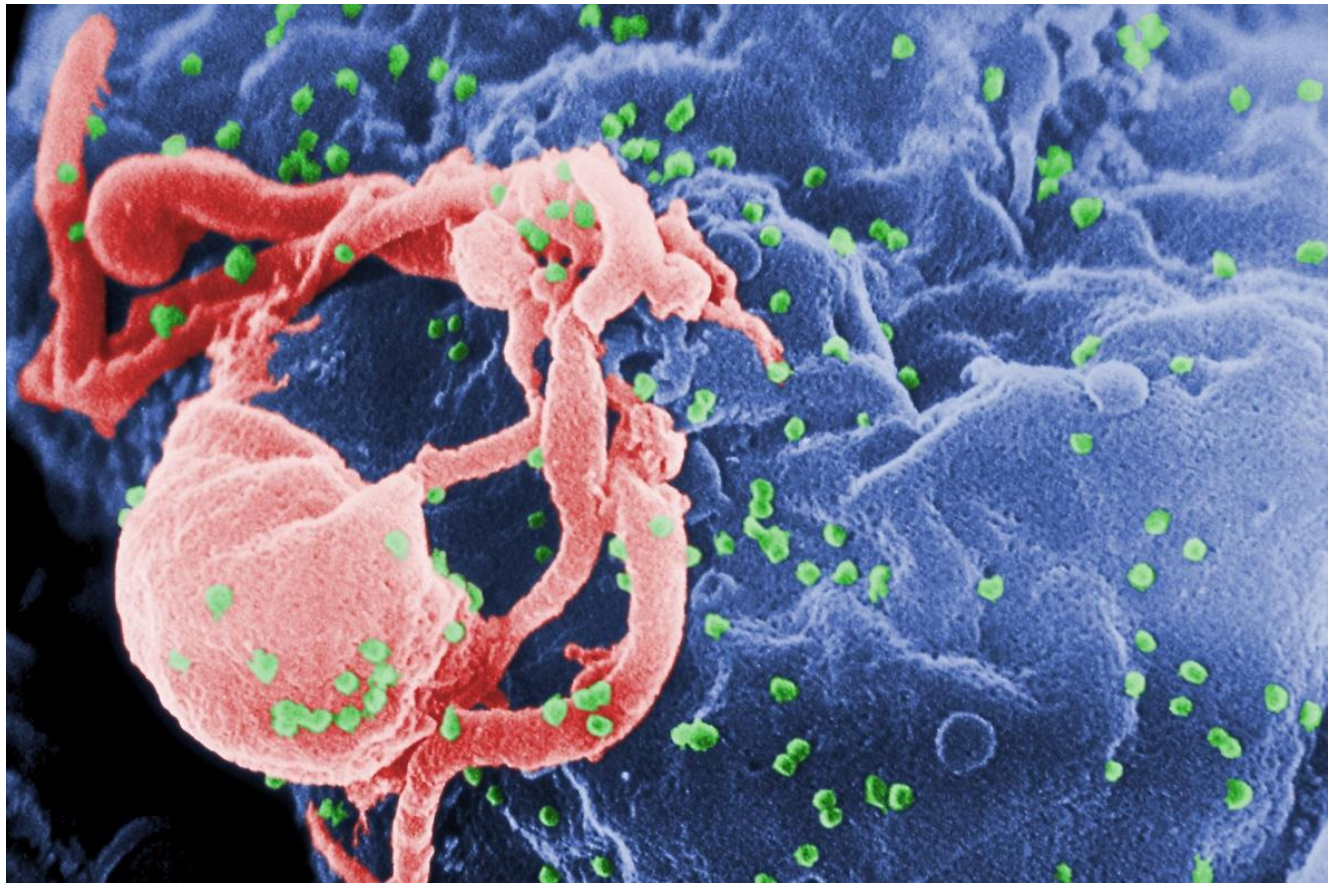
HIV Infection and AIDS: Pathogenesis 2

Uninfected, opsonized CD4+ cells are phagocytosed.

- gp120s on the surface of the one HIV particle can bind to multiple CD4s on the same cell cause crosslinking of neighboring CD4 molecules. This crosslinking triggers apoptosis. **Apoptosis is the major gp120 mechanism of CD4+ cell death in HIV infection.**
- gp120 on budding viral particles binds to CD4 on its current host cell causing the host cell plasma membrane to tear.
- Simultaneous binding of gp120 on the surface of a single budding virus to CD4s on multiple uninfected cells can cause formation of a fused mass of infected and uninfected CD4+ cells called a **syncytium**. Cell fusion and cell death follows.

HIV Infection and AIDS: Pathogenesis 3

HIV Budding From CD4+ Lymphocyte



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HIV Infection and AIDS: Pathogenesis 4

The Progression of HIV Infection

- **Incubation** occurs during the weeks or months after HIV infection, the patient is usually **asymptomatic**. The infection is not picked up by lab tests, because anti-HIV antibodies have not yet formed. The virus is rapidly replicating and killing CD4+ cells in mucosal membranes, especially those in the digestive tract.
- **Seroconversion** occurs 3 weeks to 6 months after infection when anti-HIV antibodies are detected in the blood. By this time, the patient feels ill with flu-like symptoms. A rash may be present. The CD4+ T cell count is greater than 400 cells per microliter (ul). Normal is 500-1500/ul. The CD8+ T cell count is usually elevated. Total WBC is depressed and platelets are depressed. The sed rate is high. HIV is present in high numbers in blood and genital fluids, so the person is **very infective**.

HIV Infection and AIDS: Pathogenesis 5

The Progression of HIV Infection

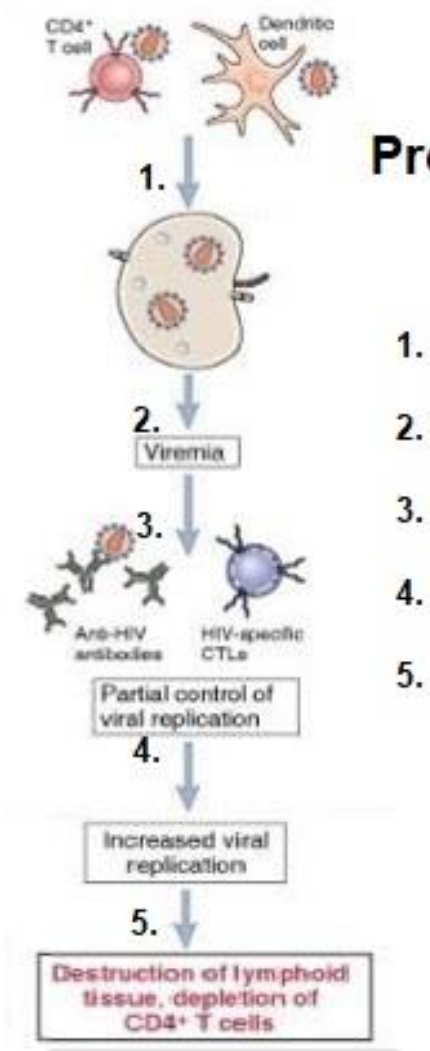
- **Latency** occurs 1 to 4 weeks after seroconversion. The symptoms disappear, but the person is still infective. HIV is in the lymphatic tissues gradually destroying them. The immune system has mounted an antiviral response, so the virus is less active. The lymph nodes are enlarged due to the immune response. The CD4+ T cell count is still above 400/ul. Latency can last anywhere from 3 to 12 years.
- **Symptomatic chronic phase**, a period of rapid viral production lasting up to 18 months, ends the latency period. The **lymph nodes have been destroyed** and the virus has escaped into the bloodstream (**viremia**). The immune system's antiviral response weakens as the CD4+ T cell count declines below 400/ul. Severe skin and mucous membrane infections occur.

HIV Infection and AIDS: Pathogenesis 6

The Progression of HIV Infection

- **AIDS** When the CD4+ T cells count falls **below 200/ul** the diagnosis of AIDS is made. By this time the person has one or more serious opportunistic infections and one or more cancers.
- In general, the progression of HIV infection in children is more rapid than that in adults.

HIV Infection and AIDS: Pathogenesis 7



Progression from HIV Infection to AIDS

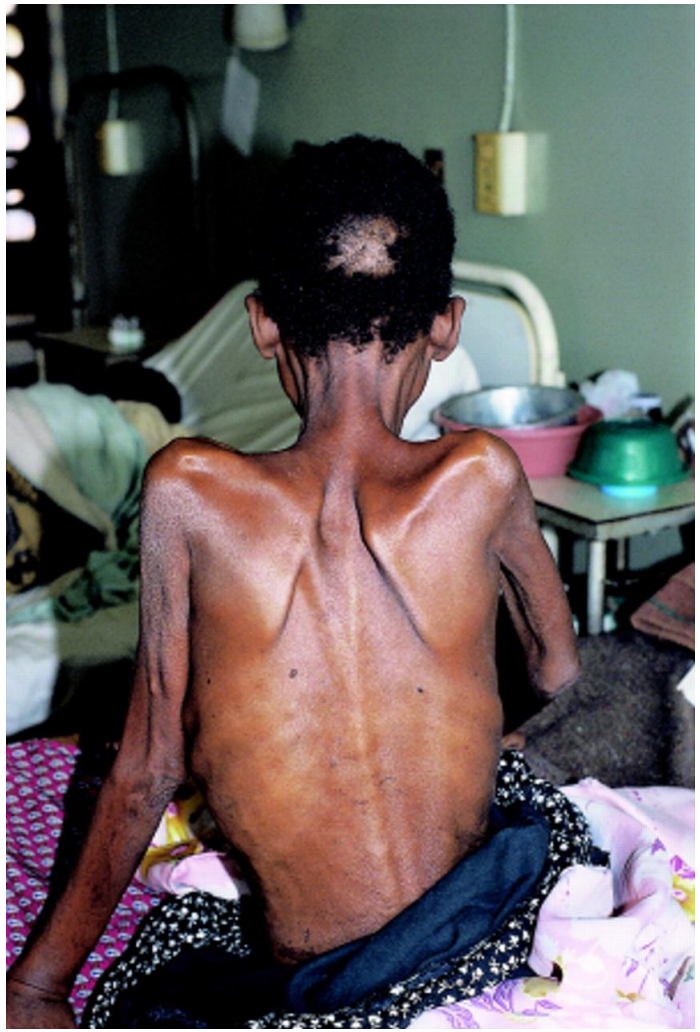
1. Infected CD4 cells invade lymphoid tissues.
2. Virus is disseminated throughout the body via blood.
3. An immune response is generated producing latency.
4. As CD4 cells are killed off, viral replication increases.
5. Depletion of CD4 cells and destruction of lymphoid tissue

HIV Infection and AIDS: Clinical Manifestations

Clinical Manifestations of HIV/AIDS

- The most significant systemic symptom is **wasting**. It is due to a combination of effects: anorexia, malabsorption and elevated metabolic rate.
- Other systemic effects: fever, chills, headache, nausea, diarrhea, vomiting, fatigue, weakness, sore throat, stiff neck, aching joints, rash
- Every body system experiences opportunistic infections.
- **Pneumocystis pneumonia** is a common killer
- **Kaposi sarcoma** is a cancer commonly associated with AIDS.
- **Neurologic** manifestations include peripheral neuropathy, dementia, headache, apathy and other mental/emotional deficits.
- **Children** with HIV experience physical and mental developmental delays.

HIV Infection and AIDS: Clinical Manifestations 1



Images from NIH image gallery

HIV Infection and AIDS: Diagnosis

HIV Testing

- HIV tests are based on the reaction between test kit antibodies that bind to anti-HIV antibodies in the patient's serum or saliva.
- An **ELISA** test for HIV uses purified HIV protein applied to plastic beads or placed in a well on a small plastic tray.
  - The subject's serum or saliva is applied to the beads or placed in the well with the HIV protein.
  - In a positive test anti-HIV antibodies from the subject's serum or saliva bind to the HIV protein.
  - Then special **antibodies direct against human antibodies** are applied. To the beads or the well.
  - If the "anti-human antibody" antibodies bind to the subject's bound anti-HIV antibodies there is a color change or fluorescence occurs.

HIV Infection and AIDS: Treatment

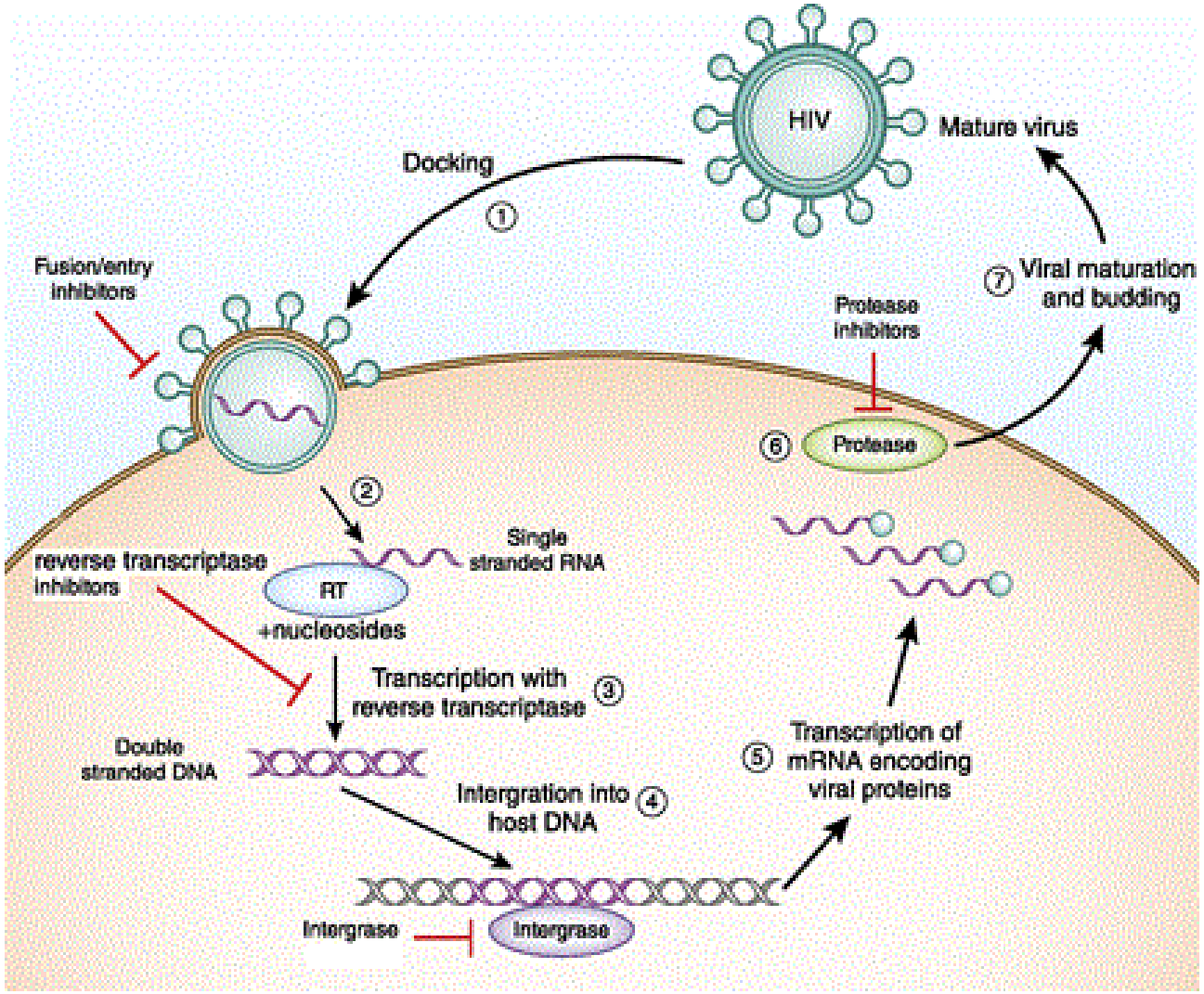
Treatment for HIV/AIDS

- **Antiretroviral drugs (multiple drugs taken together)**
  - Reverse transcriptase inhibitors block conversion of viral RNA to DNA.
  - Protease inhibitors block processing of viral proteins.
  - Integrase inhibitors block integration of viral DNA into host DNA.
  - CCR5 antagonists block binding of HIV to WBC surface.
- **Vigorous treatment of infections and malignancies**
- **Immune system boosting medications**

HIV Infection and AIDS: Treatment 1

- **Prevention strategies**
  - The development of an **HIV vaccine** has eluded scientists because the virus genetic material mutates so frequently. That is because reverse transcriptase is an **error prone enzyme** that makes frequent complementary base pairing mistakes. Therefore the antigens associated with HIV are constantly changing.
    - **PReP**=Pre-exposure Prophylaxis-individuals who are HIV-negative, but at high risk of infection take medication (Truvada) daily.
    - Truvada is a combination of two drugs used to treat AIDS. Research has shown that the PReP regimen reduces the risk of contracting HIV by up to 92% among high risk individuals.

HIV Infection and AIDS: Treatment 2



QUIZ 3CD

- COMPLETE QUIZ 3CD.
- THEN PREPARE FOR EXAM 3.

EXAM 3

- COMPLETE EXAM 3.
- THEN GO ON TO MODULE 4AB PPT.