CHAPTER 17

THE IMMUNE RESPONSE

OBJECTIVES

- Familiarize students with the basic events that occur as the body responds to the presence of an intruding microbe.
- Describe the weapons employed in the arsenal of the immune system.
- Explain what is meant by "self" and "not self".
- Delineate the elements of the innate immune response.
- Distinguish between humoral and cell-mediated immunity.
- Describe the general characteristics of the acquired immune response (humoral and cell-mediated immunity).
- Clarify the series of events that lead to the development of humoral immunity.
- Clarify the series of events that lead to the development of cell-mediated immunity.
- Elaborate the details of the Clonal Selection Theory as it relates to both T cells and B cells.
- Explain the relationship of vaccination and immunization mechanisms to the principles illustrated by the Clonal Selection Theory.
- Explain the process by which T lymphocytes are activated and their mechanism of action.
- Describe the function of cytotoxic T lymphocytes and helper T lymphocytes.
- Delineate basic antibody structure including the heavy and light chains.
- Differentiate between the structure and function of the five types of immunoglobulins.
- Explain the structure of the constant and variable portions of the heavy and light chains.
- Explain the importance and functions of the variable and constant portions of antibody heavy and light chains.
- Describe the structure and functions of the constant and variable portions of the heavy and light chains of the antibodies.
- Explain the evolutionary significance of the amino acid sequence similarities between the constant portions (and variable portions) of the antibody heavy and light chains.
- Point out the importance of the hypervariable sites to the ability of antibodies to interact with a specific antigen.
- Describe how DNA rearrangement generates a staggering diversity of antibodies with relatively few genes.
- Describe the structure, function and importance of the major histocompatibility complex proteins and the mechanisms by which they help initiate an immune response.
- Outline the process by which lymphocytes are activated, including the involvement of antigen-presenting cells.

LECTURE OUTLINE

An Overview of the Immune Response – Background Information

I. Living organisms provide ideal habitats in which other organisms (viruses, bacteria, protists, fungi, animal parasites) can grow

A. Vertebrates have evolved several mechanisms that allow them to recognize & destroy infectious agents

B. They develop immunity against pathogens - results from combined activities of many different cells

1. Some patrol body; others are concentrated in lymphoid organs (bone marrow, thymus, spleen, lymph nodes)
2. Together, these dispersed cells & discrete organs form immune system

C. Immune system (IS) cells screen body for presence of "foreign" macromolecules (different from body's normal macromolecules) —> if foreign material found, IS mounts specific, concerted attack against it

1. Implicated in body's fight against cancer; the degree to which the IS can recognize & kill cancer cells is controversial
2. IS may mount an inappropriate response that attacks body's own tissues (can lead to serious disease)

D. Weapons of the immune system

1. Cells that kill or ingest infected or altered cells
2. Soluble proteins that can neutralize, immobilize, agglutinate or kill pathogenic cells & viruses

II. Body outer surface & linings of internal tracts provide excellent barrier to penetration by pathogens (viruses, bacteria & parasites)

A. If surface barriers are breached, a series of immune responses is initiated that contain the invasion

B. Immune responses divided into two general categories - innate & adaptive (acquired) responses

1. Both depend on body's ability to distinguish between materials that are supposed to be there ("self") & those that are not (foreign or "nonself")

III. Two pathogen categories - different types of immune mechanisms have evolved to combat them

A. Those found primarily inside host cell (all viruses, some bacteria & certain protozoan parasites)

B. Those found mostly in host extracellular compartments (most bacteria, other cellular pathogens)

An Overview of the Immune Response – Innate Immune Responses

I. Innate immune responses - body mounts them immediately without requiring previous contact with the microbe; the first line of defense; characterized by lack of specificity

A. The same defensive activities are effective regardless of the pathogen presenting the threat – an invading microbe typically makes its first contact with the innate IS when it is greeted by a phagocytic cell

B. Phagocytic cells like macrophages have the function of recognizing foreign objects & sounding an appropriate alarm

1. They possess receptor proteins on their surface that recognize conserved chemical motifs that are characteristic of pathogens
2. Activation of a TLR initiates a signal cascade within the cell that can lead to a variety of protective immune responses

C. The best studied of these receptors are the Toll-like receptors (TLRs); at least 10 TLRs are expressed in humans; within this family are receptors that recognize:

1. The lipopolysaccharide or peptidoglycan components of the bacterial cell wall

2. The protein flagellin found in bacterial flagella
3. Unmethylated CpG dinucleotides, which are characteristic of bacterial DNA

II. Innate responses to an invading pathogen are typically accompanied by a process of inflammation at the site of infection where fluid, cells & dissolved substances leak out of blood & into affected tissues

A. These events are accompanied by local redness, swelling & fever

B. Inflammation provides a means for concentrating the body's defensive agents at site where needed

1. Phagocytic white cells (neutrophils, macrophages) leave bloodstream & migrate toward site of infection in response to chemicals (chemoattractants) released at the site
2. Once there, these cells recognize, engulf & destroy the pathogen

III. Blood also contains a group of soluble proteins (**complement**), which bind extracellular pathogens & destroy them; activated complement assembly perforates bacterial cell membrane causing lysis & death

IV. Responses against intracellular pathogens (viruses) are targeted mostly against already-infected cells

A. Cells infected with certain viruses are recognized by a type of nonspecific lymphocyte (natural killer [NK] cell) – they cause death of infected cells by inducing them to undergo apoptosis

B. NK cells can also kill certain types of cancer cells *in vitro* & may destroy them before a tumor forms

C. Normal (noninfected or nonmalignant) cells have surface molecules protecting them from NK cell attack

V. Another type of antiviral response is initiated within the infected cell itself - virus-infected cells make type 1 interferons (interferon α [IFN-α] & interferon β [IFN-β])

A. Interferons are secreted into the extracellular space where they bind to surface of noninfected cells, rendering them resistant to subsequent infection

B. Interferons activate a signal transduction pathway that results in phosphorylation & consequent inactivation of translation factor eIF2

C. eIF2 inactivation renders the cells unable to synthesize viral proteins required for virus replication

VI. The innate & adaptive immune systems do not function independently, but work closely together to destroy a foreign invader

A. The same phagocytic cells that activate an immediate innate response are responsible for initiating the much slower, more specific adaptive immune response

An Overview of the Immune Response – Acquired (Adaptive) Immune Responses

I. Adaptive (or acquired) immune responses – how do they differ from the innate immune responses?

A. Unlike innate responses, they require a lag period during which the immune system gears up for attack against a foreign agent

B. Unlike innate responses, they are highly specific & can discriminate between two very similar molecules

1. Blood of a person recently recovered from measles has antibodies that react with the measles virus, but not with a related virus (like the one that causes mumps)

C. Unlike the innate system, the adaptive system also has a memory, which usually means that the person will not suffer again from the same pathogen later in life

II. All animals possess some type of innate immunity against microbes & parasites, but only vertebrates are known to mount an adaptive response

III. There are two broad categories of adaptive immunity - both types are mediated by lymphocytes, which are nucleated leukocytes [white blood cells] that circulate between the blood & lymphoid organs

A. Humoral immunity - done by antibodies, which are globular, blood-borne proteins of the immunoglobulin (Ig) superfamily; humoral immunity is mediated by B lymphocytes (B cells)

B. Cell-mediated immunity - carried out by cells; mediated by T lymphocytes (T cells)

IV. Humoral immunity - when B cells are activated, they differentiate into cells that secrete antibodies (ABs)

A. ABs are directed primarily against foreign materials situated outside the body's cells; among the things that ABs attack are:

1. Protein & polysaccharide components of bacterial cell walls
2. Bacterial toxins
3. Viral coat proteins

B. How do antibodies protect the body?

1. ABs can bind to a bacterial toxin or virus particle & directly prevent it from entering host cell
2. ABs function as molecular tags, binding to invading pathogen & marking it for destruction; AB-coated bacteria are rapidly ingested by wandering phagocytes or destroyed by complement in blood
3. ABs are not effective against pathogens that are present inside cells; this is the reason for a second weapons system

V. Cell-mediated immunity - if activated, T cells can specifically recognize & kill an infected (or foreign) cell

VI. B & T cells arise from the same type of precursor cell (a **pluripotent hematopoietic stem cell**), but they differentiate along different pathways in different lymphoid organs

A. Sites of differentiation of B & T cells

1. B cells (B lymphocytes) differentiate in fetal liver or adult bone marrow
2. T cells (T lymphocytes) differentiate in thymus gland, an organ located in chest that reaches its peak size during childhood

B. Because of these differences, humoral & cell-mediated immunity can be dissociated to a large extent

1. Humans may suffer from congenital agammaglobulinemia, a rare human disease, in which humoral immunity (ABs) is deficient, while cell-mediated immunity is normal

The Clonal Selection Theory (As It Applies to B Cells): Historical Background

I. Infection with virus or exposure to foreign material soon results in a high blood concentration of ABs capable of reacting with the foreign substance, known as an **antigen** (Ag)

A. Most Ags consist of proteins or polysaccharides, but lipids & nucleic acids can also act as Ags

II. How does the body make ABs specific for a particular Ag? - at first, it was thought that Ag instructs lymphocytes to produce complementary ABs
   A. Thought that an Ag wraps itself around an AB & molds it into a shape complementary to Ag so the Ag & AB can combine
   B. In this instructive model, lymphocytes could only gain this ability to make a specific antibody after its initial contact with Ag

III. Niels Jerne (Danish immunologist, 1955) – suggested a radically different mechanism, that the body produces small amounts of randomly structured ABs in the absence of any Ag
   A. As a group, these ABs could combine with any Ag to which a person might someday be exposed
   B. When a person is exposed to Ag, it combines with a specific AB —> leads to subsequent production of that particular AB —> thus, Ag selects those preexisting ABs capable of binding to it

IV. F. MacFarlane Burnet (Australian immunologist, 1957) - proposed comprehensive AB formation model, expanding on Jerns's idea
   A. This **clonal selection theory** is virtually completely accepted; it is compatible with all evidence collected since its proposal

The Clonal Selection Theory (As It Applies to B Cells): The Process

I. Each B cell becomes committed to produce one species of AB
   A. B cells arise from a population of undifferentiated & indistinguishable progenitor (stem) cells
      1. As it differentiates, a B cell becomes committed as a result of DNA rearrangements to producing only one species of AB molecule
      2. Thousands of different DNA rearrangements are possible so that different B cells make different ABs
   B. B cells may appear identical in microscope, but they can be distinguished by the ABs they produce

II. B cells become committed to AB formation in the absence of Ag
   A. The whole repertoire of AB-producing cells a person will ever have is already present in lymphoid tissue before stimulation by an Ag & is independent of the presence of foreign materials
   B. Each B cell displays its particular AB on its surface with the Ag-reactive portion facing outward
      1. B cells are coated with Ag receptors that bind specifically with Ags having complementary structure
      2. Although, most lymphoid cells are never required during a person's lifetime, the IS is primed to respond immediately to any Ag to which a person may be exposed

III. AB production follows selection of B cells by Ag - B cell activation usually requires T cell involvement
   A. Some Ags, however, (like bacterial cell wall polysaccharides) activate B cells by themselves (**thymus-independent Ags**)

B. Example: expose person to *Haemophilus influenzae* type B (encapsulated bacterium; can cause fatal meningitis) —> capsule contains polysaccharide that can bind a tiny fraction of body's B cells
   1. B cells that bind polysaccharide contain membrane-bound ABs whose combining site allows them to interact specifically with that Ag; Ag selects lymphocytes that make ABs that bind that Ag
   2. Ag binding activates B cell, causing it to proliferate & form a population (**clone**) of B lymphocytes that all make the same AB
   3. Some of the activated cells differentiate into short-lived **plasma cells** —> secrete large amounts of ABs
   4. Unlike their precursors, plasma cells have the extensive rough ER characteristic of cells that are specialized for protein synthesis & secretion

IV. Immunologic memory provides long-term immunity – not all B cells in clone become plasma cells; some cells remain in lymphoid tissue as **memory cells** (respond rapidly at a later date if Ag reappears in body)
   A. Plasma cells die off after Ag stimulus removal, but memory cells may persist for a person's lifetime
   B. When stimulated by the same Ag, some memory cells proliferate rapidly into plasma cells in a matter of hours rather than the days needed for first response (**secondary immune response**)
   C. Thus, we only suffer once in a lifetime from effects of a particular viral or bacterial strain

V. Immunologic tolerance prevents the production of ABs against self – genes encoding antibodies are generated by a process in which DNA segments are randomly combined
   A. As genes are rearranged during B cell development, the body invariably makes genes encoding ABs that react with body's own tissues —> could cause widespread organ destruction & subsequent disease
      1. It is in the best interest of the body to prevent production of these autoantibodies (autoABs)
      2. During IS development, cells able to produce autoABs are either destroyed or rendered inactive
      3. They are destroyed by apoptosis (**clonal deletion**) or become inactivated & unable to respond to Ag (**clonal anergy**)
   B. The body thus develops an immunologic tolerance to itself, but many cells capable of making autoABs normally remain in body
      1. A breakdown of the tolerant state can lead to development of debilitating autoimmune diseases
      2. Examples of autoimmune diseases are lupus erythematosus & rheumatoid arthritis

Vaccination

I. Vaccination - Edward Jenner (English physician, 1796) found that cowpox infection at early age protects person against deadly smallpox; noticed that milkmaids were typically spared the ravages of smallpox
   A. Cowpox (harmless; got it from cows) causes blisters as does smallpox, but they are localized & disappear; cause nothing more serious than a scar; concluded milkmaids were immune & did risky experiment
      1. Infected 8-year-old boy with cowpox & allowed time for his recovery

2. Six weeks later, Jenner intentionally infected him with smallpox by injecting him with pus from a smallpox lesion directly under his skin —> no smallpox & no signs of disease

B. Within a few years, **vaccination** had saved many lives ("vacca" – Latin word for "cow"); people intentionally infected themselves with cowpox

C. Jenner's experiment was successful because the immune response generated against the virus that causes cowpox happens to be effective against the closely related virus that causes smallpox

II. Most modern vaccines contain **attenuated pathogens**, pathogens that are capable of stimulating immunity but have been genetically crippled so that they are unable to cause disease

III. Jenner's vaccination produces immunity by exciting T cells; most others are B-cell vaccines (ex.: tetanus)

A. Tetanus – an anaerobic soil bacterium *Clostridium tetani* infection; enters the body by puncture wound

1. As they grow, the bacteria produce a powerful neurotoxin that blocks transmission across motor neuron inhibitory synapses —> results in sustained muscle contraction & asphyxiation
2. At 2 months of age, most infants are immunized by inoculation with a modified, harmless version of tetanus toxin (a **toxoid**)
3. Tetanus toxoid binds to B cells whose membrane-bound ABs have a complementary binding site
4. These B cells proliferate to form a clone of cells that make ABs that bind to the actual tetanus toxin
5. Initial response wanes, but person is left with memory cells that respond fast if exposed to toxin again

B. Immunity to tetanus does not always last a whole lifetime, so get booster shot every 10 years or so

1. Booster shot contains toxoid protein & stimulates production of additional memory cells
2. If get puncture wound 10-15 years after last booster or if one cannot recall booster, new booster is not enough —> person may be given passive immunization (inject ABs that bind to tetanus toxin)
3. Passive immunization is only effective for short time & gives no protection against later infection

T Lymphocytes: Activation and Mechanism of Action

I. T cells are also subject to the process of clonal selection – they possess a cell surface protein (T-cell receptor) that allows a specific interaction with a particular Ag

A. T-cell receptors, like B cells, are a large molecule population with differently shaped combining sites

1. Each T cell has only a single species of T-cell receptor (just like B cells that make only one type of AB)
2. Estimate that adult humans have ~10^{12} T cells that collectively show >10^{7} different Ag receptors

B. T cells are activated by Ag fragments displayed on other cells' surfaces (Ag-presenting cells, APCs)
1. Different from B cells which are activated by soluble, intact antigens

C. What happens with liver or kidney cell infected with virus? - infected cell displays portions of viral proteins on its surface
1. Infected cell then binds to a T cell with appropriate T-cell receptor, alerting IS about pathogen entry

II. Any infected cell can act as an APC in activating T cells, but some cells are specialized for this function

A. Dendritic cells (DCs) & macrophages - professional APCs (play key roles in initiating immune response)
1. DCs are often described as sentinels of IS - they stand guard at sites in peripheral tissues where pathogens are likely to enter body (skin, airways)
2. They are particularly adept at initiating an adaptive immune response

B. When present in body's peripheral tissues, immature DCs recognize & internalize microbes & other foreign materials by phagocytosis
1. Once microbe is inside DC, it must be processed before its components can be presented to another cell
2. Ag processing requires that the ingested material is fragmented enzymatically in cytoplasm —> then the fragments are moved to cell surface

C. DCs that have processed Ag go to nearby lymph nodes where they differentiate into mature APCs

D. Once in lymph nodes, DCs come into contact with a large resident T-cell pool, including a minute percentage whose T-cell receptors bind specifically to the processed foreign Ag, activating T cell
1. Activated T cells proliferate to form a clone of cells with same the T-cell receptor
2. It is estimated that a single activated T cell can divide 3 – 4 times per day for several days, generating a tremendous T-cell population capable of interaction with the foreign Ag
3. The massive proliferation of specific T lymphocytes in response to infection is often reflected in enlargement of local lymph nodes
4. Once foreign Ag has been cleared, the vast majority of the expanded T-cell population dies off by apoptosis, leaving behind a relatively small population of memory T cells
5. The memory cells are capable of responding rapidly after future contact with the same pathogen

III. T cells function at close range by interacting directly with other cells, including APCs, B cells, other T cells or target cells located anywhere in the body, unlike B cells, which secrete antibodies

A. This cell-cell interaction may lead to the activation, inactivation or death of the other cell

B. In addition to direct cell contact, many T-cell interactions are mediated by highly active chemical messengers (**cytokines; CKs**) that work at very low concentrations
1. CKs - small proteins (interferons [IFNs], interleukins [ILs], tumor necrosis factors [TNFs]) made by a wide variety of cells
2. CKs bind specific receptors on surface of responding cell & generate an internal signal that alters the cell's activity
3. In response to CK, a cell may prepare to divide, differentiate or secrete its own CKs

C. One family of small CKs (**chemokines**) acts primarily as chemoattractants that stimulate migration of lymphocytes into inflamed tissue
1. Different types of lymphocytes & phagocytes possess receptors for different chemokines
2. Thus, their migration patterns can be separately controlled

D. Two major T cell subclasses (cytotoxic T lymphocytes & helper T lymphocytes) can be distinguished by proteins on their surfaces & biological functions
1. A third class of T cells known as suppressor (or regulatory) T cells is thought to suppress other T cells capable of mounting an autoimmune response

IV. Cytotoxic T lymphocytes (CTLs or T_C cells) - screen body cells for abnormalities
A. Under normal circumstances, healthy cells are not harmed by CTLs, but aged or infected cells, & maybe malignant cells, are attacked & killed; CTLs kill target cells by inducing them to undergo apoptosis
B. Two distinct killing pathways have been discovered: the release of perforins & granzymes into the space between the cells and apoptosis
1. The perforin-granzyme pathway - CTL releases perforins & granzymes into the space between cells
a. Perforins - proteins that assemble within target cell membrane to form transmembrane channels
b. Granzymes are proteolytic enzymes that enter the perforin channels & activate caspases, which are proteolytic enzymes that initiate the apoptotic response
2. Alternate pathway - CTL cell binds to receptor on target cell surface, activating a suicide pathway in the target cell
C. By killing infected cells, CTLs eliminate viruses, bacteria, yeast, protozoa & parasites after they have gotten into host cells, where they are no longer accessible to circulating ABs
D. CTLs possess a surface protein called CD8 (***c***luster ***d***esignation ***8***) & so are often called $CD8^+$ cells

V. Helper T lymphocytes (T_H cells) - regulatory cells, not killers; distinguished from CTLs by presence of CD4 protein on cell surface instead of CD8
A. 2 major helper T cell classes: T_H1 & T_H2, which are distinguished by the CKs they secrete & their basic function; the 2 types differentiate from a common precursor after stimulation by different CKs
1. T_H1 cells produce IFN-γ & they protect the body against intercellular pathogens by activating macrophages to kill pathogens they might harbor
2. T_H2 cells produce other interleukins, like IL-4 & they protect against extracellular pathogens by activating B cells to produce ABs
B. T_H cells are activated by professional APCs (macrophages, DCs) - one of the first & most important steps in initiating an adaptive immune response
1. Once activated, T_H cells regulate subsequent immune responses by recognizing & activating other lymphocytes that are specific for the same Ag
C. Nearly all B cells require the help of T_H cells before they can mature & differentiate into AB-secreting plasma cells - B cells are activated by direct interaction with a T_H cell
1. Thus, AB formation requires activation of both B & T cells that interact specifically with same Ag
2. A few Ags (thymus-independent) elicit AB formation by B cells without T cells (large polymeric molecules with repeating substructures, lipopolysaccharide), a bacterial cell wall component
D. T_H cells are the primary targets of HIV; it enters host cell by binding to its CD4 surface protein
1. Most HIV-infected people stay free of symptoms as long as their T_H-cell counts are relatively high (>500 cells/µl)
2. Once count drops below ~200 cells/µl (normal count is >1,000 cells/µl) —> person develops full-blown AIDS & becomes prone to attack by viral & cellular pathogens

Selected Topics on the Cellular & Molecular Basis of Immunity: Antibody Structure

I. ABs are proteins made by B cells & their descendants (plasma cells)
   A. B cells incorporate ABs into their plasma membranes, where they serve as Ag receptors
   B. Plasma cells secrete these proteins into the blood or other bodily fluids, where they serve as a molecular arsenal in the body's war against invading pathogens
      1. Interactions between soluble ABs & Ags on the surface of a virus or bacterial cell can neutralize the pathogen's ability to infect a host cell
      2. The interactions also facilitate the pathogen's ingestion & destruction by wandering phagocytes
   C. The IS produces millions of different AB molecules that taken together can bind any type of foreign substance to which the body may be exposed
      1. Though IS shows great diversity in the ABs it can produce, a single AB can interact with only one or a few closely related antigenic structures

II. ABs are globular proteins with a modular structure & are called **immunoglobulins** (Igs); they are built of 2 types of polypeptide chains linked to one another in pairs by disulfide bonds
   A. Larger heavy chains (molecular mass of 50,000 - 70,000 daltons)
   B. Smaller light chains (molecular mass of 23,000 daltons)

III. There are 5 different classes of Ig (IgA, IgD, IgE, IgG, IgM) that have been identified - different Igs appear at different times after exposure to a foreign substance & have different biological functions
   A. IgMs are first ABs secreted by B cells after Ag stimulation; appear in blood after a lag of a few days; have a relatively short half-life (~5 days)
   B. IgM appearance is followed by secretion of longer-lived IgGs and/or IgEs
      1. IgGs are the predominant ABs found in blood & lymph during a secondary response to most Ags
      2. IgEs are made at high levels in response to many parasitic infections & also bound with high affinity to mast cell surface, triggering histamine release —> inflammation & symptoms of allergy
   C. IgAs are the predominant AB in secretions of respiratory, digestive & urogenital tracts
   D. IgD function is unclear

IV. Two types of light chains: kappa (κ) & lambda (λ); both are present in the Igs of all 5 classes; heavy chains are unique to each class & define them - example: IgGs
   A. IgGs are made of 2 identical heavy & 2 identical light chains arranged to form Y-shaped molecule
      1. Humans actually make 4 related heavy chains as part of IgG molecules (IgG1, IgG2, IgG3, IgG4)
   2. Humans make 2 related heavy chains as part of their IgA molecules (IgA1, IgA2)

V. Tried to determine basis of AB specificity by purifying ABs & finding amino acid sequence of a number of specific ABs; hard since blood is filled with 1000s of very similar ABs too similar to be separated

A. Problem was solved when it was discovered that the blood of patients suffering from multiple myeloma (a type of lymphoid cancer) contained large quantities of a single AB molecule
   1. Cancer is a monoclonal disease; cells of tumor arise from the proliferation of a single wayward cell
   2. So a patient produces large amounts of the specific AB made by the particular cell that became malignant; each patient makes one highly abundant AB species
   3. Another patient produces lots of another AB; with many patients, get lots of many different ABs

B. Purified ABs from multiple patients; compared their amino acid sequences —> saw important pattern
   1. Half of each kappa light chain (110 aminos at N terminus) had constant amino acid sequence (C_L) among all kappa chains; the other half varied patient to patient (V_L)
   2. Similar comparison of sequences of several lambda chains from different patients showed that they consist of a section of constant sequence & a section whose sequence varied from one Ig to next
   3. Heavy chains also contained variable (V) & constant (C) portion; only one quarter of each heavy chain is variable (V_H); other three-quarters of heavy chain is constant (C_H)

C. Heavy chain constant portion can be divided into 3 sections of approximately equal length that are clearly homologous to one another; they are designated C_H1, C_H2, C_H3
   1. It appears that the 3 parts of the constant portion of the IgG heavy chain arose during evolution by duplication of an ancestral gene that coded for an Ig unit ~110 amino acids long
   2. This is also true of C portions of other Ig heavy chain classes & C portions of both κ & λ light chains
   3. Variable (V_H & V_L) regions are also thought to have arisen by evolution from same ancestral Ig unit
   4. Each of the homologous Ig units of a light or heavy chain folds independently to form a compact domain that is held together by a disulfide bond
   5. In an intact Ig molecule, each light chain domain associates with a heavy chain domain; each domain is encoded by its own exon

D. Specificity of AB is determined by amino acids of the Ag-combining sites at the ends of each arm of the Y-shaped AB – the 2 binding sites of a single IgG are identical

1. Each binding site is formed by the association of the variable portions of a light & a heavy chain
   2. Assembly of ABs from different combinations of light & heavy chains allows production of a tremendous variety of ABs from a modest number of different polypeptides

E. Variable portions of both heavy & light chains contain subregions that are especially variable (**hypervariable**; Hv) from one AB to another; accounts for great diversity of AB specificity
   1. Light & heavy chains both contain 3 hypervariable stretches clustered at the ends of each arm on the AB molecule; they play a prominent role in forming the structure of the Ag-combining site

2. Ag-combining site can range from a deep cleft to a narrow groove or relatively flat pocket
   3. Hypervariable region amino acid sequence variations account for great diversity of AB specificity, allowing these molecules to bind Ags of every conceivable shape

VI. Functions of variable & constant variable portions of ABs

A. AB combining site has a complementary stereochemical structure to a particular Ag portion (the **epitope** or **antigenic determinant**)
   1. Because of their close fit, ABs & Ags form stable complexes, even though they are joined only by noncovalent forces that are individually quite weak

2. The 2 hinge regions within the AB molecule provide the flexibility necessary for the AB to bind to 2 separate antigen molecules or to a single molecule with 2 identical epitopes

B. Hypervariable regions of light & heavy chains determine an AB's combining site specificity
   1. The remaining portions of the variable domains provide a scaffold that maintains the overall combining site structure

C. Constant portions of ABs are also important - different AB classes have different heavy chains whose constant regions differ a lot in length & sequence —> accounts for their different biological functions
   1. IgM heavy chains bind & activate one of the complement system proteins —> leads to lysis of the bacterial cell to which the IgM molecules are bound
   2. IgE heavy chains play an important role in allergic reactions by binding to specific receptors on mast cell surfaces, thus triggering histamine release
   3. IgG heavy chains bind specifically to macrophage & neutrophil surface receptors —> induce these phagocytes to ingest the particle to which the ABs are bound
   4. IgG heavy chains also allow these ABs to go from mother's blood vessels to those of fetus during pregnancy —> provides passive immunity to infectious organisms for a fetus or newborn

D. But the ability of maternal ABs to get into the fetus can also lead to a life-threatening condition called erythroblastosis fetalis

1. An Rh^- mother must have had a child with Rh^+ phenotype (Rh^+/Rh^-) during a previous pregnancy
2. The mother is usually exposed to Rh^+ fetal Ag during delivery of first child, who is not affected
3. If mother has another Rh^+ pregnancy, her blood ABs can enter fetal circulation —> kill fetal RBCs
4. Babies born with this condition are given blood transfusion that cleanses blood of maternal ABs

Selected Topics on the Cellular & Molecular Basis of Immunity: DNA Rearrangement of Genes Encoding B- and T-Cell Antigen Receptors

I. William Dreyer (Caltech, 1965) & J. Claude Bennett (Univ. of Alabama) - proposed "two gene - one polypeptide" hypothesis to account for antibody structure with combination of variable & constant regions

A. Proposed that each antibody chain is encoded by 2 separate genes - a C gene & a V gene that somehow combine to form one continuous "gene" coding for a single heavy or light chain

B. Susumu Tonegawa (Basel, Switzerland; 1976) - provided evidence in favor of DNA rearrangement
   1. Compared DNA length between sequences coding for C & V parts of a specific AB chain in 2 different mouse cell types - early embryonic & malignant AB-producing myeloma cells
   2. DNA segments encoding C & V portions of AB were widely separated in embryonic DNA but very close together in DNA obtained from AB-producing myeloma cells
   3. Strongly suggested that DNA segments encoding parts of ABs became rearranged during formation of AB-producing cells

II. DNA sequences involved in formation of human κ light chains are located on chromosome 2 (λ light chain genes are on chromosome 22 & heavy chains are encoded on chromosome 14)

A. Research revealed the precise arrangement of DNA sequences that give rise to antibody genes - organization of human κ light chain DNA sequences in the germ line DNA (sperm or egg)
   1. A variety of different V_K genes seen in linear array, separated from a single C_K gene by some distance
   2. Nucleotide sequence analysis of V genes indicated they are shorter than required to encode the V region of the κ light chain
   3. Nucleotide stretch encoding the 13 amino acids at V_K region C-end is found at some distance from the rest of V_K-gene sequence; nucleotides encoding the C-terminus of V region is called **J segment**
   4. There are 5 distinct J_K segments of related DNA sequence arranged in tandem
   5. The J_K segment cluster is separated from the C_K gene by an additional stretch of >2,000 nucleotides

B. A complete κ V gene is formed as a specific V_K gene is joined to one of the J_K segments with the intervening DNA excised; process is catalyzed by a protein complex called **V(D)J recombinase**
   1. The V_K sequence generated by this rearrangement is still separated from C_K gene by 2,000-4,000 bps
   2. No further DNA rearrangement occurs in κ gene assembly prior to transcription
   3. The entire genetic region is transcribed into a large primary transcript from which introns are excised by RNA splicing

III. To begin rearrangement, double stranded cuts are made in the DNA between a V gene & a J gene
   A. The cuts are catalyzed by a pair of proteins called RAG1 & RAG2 that are part of V(D)J recombinase
   B. The 4 free ends formed are then joined in such a way that the V & J coding segments are linked to form an exon that encodes the variable region of the polypeptide chain
   C. The 2 ends of the intervening DNA are linked to form a small circular piece of DNA that is displaced from the chromosome
   D. Joining of the broken DNA ends is accomplished by the same basic process used to repair DNA strand breaks

IV. Ig DNA sequence rearrangement has important consequences for a lymphocyte – chief one is variability; such variability can be generated in a few different ways
   A. Once a specific V_K sequence is joined to a J_K sequence —> no other species of κ chain can be synthesized by that cell
      1. The DNA of human germ cells is estimated to contain ~40 functional V_K genes
      2. If we assume that any V sequence can join to any J sequence —> a person can make ~200 different κ chains (5 J_K segments **x** 40 V_K genes)
   B. The site at which a J sequence joins a V sequence can vary somewhat from one rearrangement to another
      1. Thus, the same V_K & J_K genes can be joined in 2 different cells to produce κ light chains having different amino sequences
   C. Even more variability is achieved by deoxynucleotidyl transferase, which inserts nucleotides at sites of strand breakage
   D. The above sources of additional variability (B & C) increase κ chain diversity an additional 10-fold, bringing the number to at least 2,000 species
   E. The site at which V & J sequences are joined is part of one of the hypervariable regions of each AB polypeptide; thus, slight differences at a joining site can have important effects on the AB-Ag interaction

V. Similar types of DNA rearrangement occur during commitment of a cell to the synthesis of a particular λ light chain & to a particular heavy chain

A. Heavy chain variable regions form from 3 distinct segments (V, D & J) by similar rearrangements

1. The human genome has 51 different functional V_H segments, 25 D_H segments & 6 J_H segments
2. Given the additional diversity stemming from the variability in V_H-D_H & D_H-J_H joining, a person can synthesize at least 100,000 different heavy chains

B. The Ag receptors of T cells (TCRs) also consist of a type of heavy & light chains, whose variable regions are formed by a similar process of DNA rearrangement

C. The formation of AB genes by DNA rearrangement illustrates the potential of the genome to engage in dynamic activities

1. Because of this rearrangement mechanism, a handful of DNA sequences present in the germ line can give rise to a remarkable diversity of gene products
2. A person makes roughly 2000 different species of κ light chains & 100,000 different species of heavy chains
3. If any κ light chain can combine with any heavy chain —> a person can theoretically produce >200 million different species of ABs from a few hundred genetic elements in germ line
4. A person can also generate a roughly comparable number of λ chain-containing ABs

D. In summary, antibody diversity arises from:

1. The presence of multiple V exons, J exons & D exons in the DNA of the germ line
2. Variability in V-J & V-D-J joining
3. The enzymatic insertion of nucleotides

VI. Another mechanism for generating AB diversity occurs long after DNA rearrangement is done (**somatic hypermutation**)

A. When a specific Ag is reintroduced into an animal after a period of time —> ABs made during the secondary response have a much greater affinity for the Ag than those made during primary response

1. Increased affinity is due to small changes in amino acid sequence of heavy & light chain variable regions; sequence changes result from mutations in genes encoding these polypeptides
2. Rearranged DNA elements encoding AB V regions are estimated to have a mutation rate 10^5 times greater than that of other genetic loci in same cell

B. The mechanism leading to the higher V-region mutation level is unknown, but is thought to involve some kind of error-prone DNA repair; included in the mechanism are:

1. An enzyme, known as cytosine deaminase, that converts cytosine residues in DNA into uracil residues
2. One or more translesion DNA polymerases that tend to make errors when DNA containing uracils is copied or repaired

C. B cells whose mutated genes produce Ig molecules with greater antigen affinity are preferentially selected after Ag reexposure

1. Selected cells proliferate to form clones that undergo additional rounds of somatic mutation & selection, whereas nonselected cells that express low affinity Igs undergo apoptosis
2. In this way, the AB response to recurrent or chronic infections improves markedly over time

VII. Once a cell is committed to form a specific AB, it can switch the Ig class (e.g., from IgM to IgG) it produces by changing the heavy chain the cell makes; this process is known as **class switching**

A. It occurs without changing the combining site of the ABs synthesized

B. The 5 genes encoding the heavy chain constant regions (C_H portions) are clustered together in a complex

1. Class switching is done by moving a different C_H gene next to the VDJ gene that was formed previously by DNA rearrangement

C. Class switching is under the direction of cytokines secreted by helper T cells during their interaction with the B cell producing the AB molecule

1. A helper T cell that secretes IFN-γ induces a switch in the adjacent B cell from IgM synthesis to one of the IgG classes

D. Class switching allows a B cell lineage to keep making ABs having the same specificity, but different effector functions

Selected Topics on the Cellular & Molecular Basis of Immunity: Membrane-Bound Antigen Receptor Complexes

I. Ag recognition by B & T lymphocytes occurs at cell surface

A. An Ag receptor on a B cell (B-cell receptor or BCR) consists of a membrane-bound Ig that binds selectively to a portion of an intact Ag (i.e., the epitope)

B. The Ag receptor on a T cell (T-cell receptor or TCR) recognizes & binds to a small fragment of an Ag, typically a peptide ~7 – 25 amino acids in length that is held at the surface of another cell

II. Both types of Ag receptors are part of a large membrane-bound protein complex that include invariant proteins; the invariant polypeptides associated with BCRs & TCRs play a key role in signal transduction

A. The signals transmitted lead to changes in the activity of the B cell or T cell

III. Each TCR subunit contains 2 Ig-like domains, indicating that they share a common ancestry with BCRs

A. Like the heavy & light chains of the Igs, one of the Ig-like domains of each TCR subunit has a variable amino acid sequence; the other Ig-like domain has a constant amino acid sequence

B. X-ray crystallography studies show that the 2 Ag receptor types also share a similar 3D shape

Selected Topics on the Cellular & Molecular Basis of Immunity: The Major Histocompatibility Complex — Background Information

I. Early 20th century success with blood transfusions led to attempts at skin grafts between individuals

A. Tested during World War II on military personnel who had received serious burns —> the grafts were rapidly & completely rejected

B. After the war, researchers set out to determine the basis of the tissue rejection

C. It was found that skin could be grafted successfully between mice of the same inbred strain, but that grafts between mice of different strains were rapidly rejected

1. Mice of the same inbred strain are like identical twins; they are genetically identical

II. Later studies revealed that the genes governing tissue graft rejection were clustered in a region of the genome that was named the **major histocompatibility complex** (**MHC**)

A. MHC consists of ~15 different genes, most of which are highly polymorphic; >500 different alleles of MHC genes have been identified, far more than any other loci in the human genome
   1. Therefore, it is very unlikely that 2 individuals in a population have same MHC allele combination
   2. That is why transplant recipients reject transplants so easily & why they are given drugs (like cyclosporin A)

B. Cyclosporin A is required to suppress the immune system after transplant surgery
   1. It is a cyclic peptide produced by a soil fungus & inhibits a particular phosphatase in the signaling pathway leading to the production of cytokines needed for T-cell activation
   2. Such drugs help prevent rejection, but make patients susceptible to opportunistic infections similar to those that strike people with immunodeficiency diseases (like AIDS)

C. They did not evolve to prevent indiscriminate tissue transplants, so what is natural MHC protein function? – long after discovery as transplantation Ags, they were shown to be involved in Ag presentation

III. T cells are activated by an Ag that has been dissected into small peptides that are displayed on an APC surface; the Ag fragments are held at an APC surface in the grip of MHC proteins

A. Each species of MHC molecule can bind a large number of different peptides that share structural features, allowing them to fit into its binding site

B. Example: all peptides that can bind to a protein encoded by particular MHC allele, like HLA-B8, may have a specific amino acid at a certain position that allows it to fit into the MHC groove

IV. Given that each individual expresses many different MHC proteins & each MHC variant may be able to bind many different peptides, a DC or macrophage should be able to display a vast array of peptides

A. At same time, not every person can present every possible peptide in an effective manner
   1. This is thought to be a major factor in determining differences in susceptibility in a population to different infectious diseases, including AIDS
   2. HLA-DRB1*1302 allele correlates with resistance to a certain malaria type & hepatitis B infection

B. The MHC alleles present in a given population have been shaped by natural selection
   1. Those persons who possess MHC alleles that are best able to present peptides of a particular infectious agent will be the most likely to survive infection by that agent
   2. Conversely, people lacking such alleles are more likely to die without passing their alleles to offspring
   3. Thus, populations are more resistant to diseases to which their ancestors were routinely exposed
   4. This may explain why Native American populations have been devastated by certain diseases (like measles) that produce only mild symptoms in people of European ancestry

V. Entire process of T-cell-mediated immunity rests on the basis that small peptides derived from pathogen proteins differ in structure from those derived from host proteins

A. Thus, one or more peptides held at APC surface serve as a small representation of the pathogen, giving IS cells a glimpse of the type of pathogen hidden within the infected cell's cytoplasm

B. Nearly any cell in body can act as APC
   1. Most cells present Ag as incidental activity that alerts the IS to the presence of pathogen
   2. Some professional APCs (DCs, macrophages, B cells) are specialized for this function

VI. When a T cell interacts with an APC, T cells with appropriate TCRs dock onto MHC molecules projecting from the APC's surface
   A. Interaction brings TCR of T cell into an orientation that allows it to recognize specific peptide displayed within a groove of an MHC molecule
   B. MHC protein-TCR interaction is strengthened by additional contacts that form between cell-surface components (as occur between CD4 or CD8 molecules on T cell & MHC proteins on APC)

VII. MHC proteins can be subdivided into 2 major groups: MHC class I & MHC class II molecules; they have somewhat different structures & functions
   A. MHC class I molecules - made of 1 polypeptide chain encoded by an MHC allele (known as the heavy chain) associated noncovalently with a non-MHC polypeptide **(β_2-microglobulin**)
      1. Differences in heavy chain amino acid sequence are responsible for dramatic changes in the shape of the molecule's peptide binding groove
   B. MHC class II molecules - also consist of heterodimer, but both subunits are encoded by MHC alleles

VIII. Both MHC classes & β_2-microglobulin have Ig-like domains & are thus members of Ig superfamily
   A. Whereas most body cells express MHC class I molecules on their surface, MHC class II molecules are expressed primarily by professional APCs
   B. The 2 MHC classes display Ags that originate from different sites in the cell, although some overlap has been reported
      1. MHC class I molecules are primarily responsible for displaying Ags that originate within cell cytoplasm (**endogenous proteins**)
   2. MHC class II molecules mostly display exogenous Ag fragments taken into cell by phagocytosis

Selected Topics on the Cellular & Molecular Basis of Immunity: The Major Histocompatibility Antigens — Mechanism of Action in Antigen Processing

I. Processing of class I MHC-peptide complexes - Ags located in cytosol of APC are degraded into short peptides by proteases that are part of the cell's **proteasomes**
   A. These proteases cleave cytosolic proteins into fragments ~8 - 10 residues long; they are suitable for binding within a groove of an MHC class I molecule
   B. The peptides are then transported across the RER membrane & into its lumen by a dimeric protein (TAP; ***t***ransporter ***a***ssociated with antigen ***p***rocessing)
   C. Once in the ER lumen, the peptide can bind to a newly synthesized MHC class I molecule, which is an integral membrane protein of ER membrane
   D. The MHC-peptide complex moves through the biosynthetic pathway until it reaches the plasma membrane where the peptide is displayed

II. Processing of class II MHC-peptide complexes - MHC class II molecules are also synthesized as RER membrane proteins, but are joined noncovalently to a protein called Ii (blocks MHC peptide-binding site)
   A. Following its synthesis, MHC class II-Ii complex moves out of ER along the biosynthetic pathway, directed by targeting sequences located within cytoplasmic domain of Ii
   B. MHC class I & II molecules may separate from one another in the *trans* Golgi network (TGN), the primary sorting compartment along the biosynthetic pathway

1. An MHC class I-peptide complex is directed toward cell surface
2. An MHC class II-Ii complex is directed into an endosome or lysosome where the Ii protein is digested by acid proteases

C. An MHC class II molecule is then free to bind to peptides digested from Ags that were taken into the cell & directed along the endocytic pathway
1. Peptides formed in lysosomes & attached to MHC class II proteins tend to be longer (10 - 25 residues) than those formed in proteasomes & attached to MHC class I proteins (8 – 10 residues)

D. The MHC class II-peptide complex is then moved to plasma membrane, where it is displayed

III. Once on the surface of APC, MHC molecules direct the cell's interaction with different types of T cells

A. Cytotoxic T lymphocytes (CTLs) recognize their Ag in association with MHC class I molecules (**MHC class I - restricted**)
1. Normally, body cells that contact cytotoxic T cells place fragments of their own normal proteins along with MHC class I proteins —> normal cells displaying normal fragments ignored by T cells
2. Normal cells displaying normal protein fragments are ignored since T cells able to bind with high affinity to peptides derived from normal cell proteins are eliminated as they develop in thymus
3. In contrast, an infected cell displays viral protein fragments attached to MHC class I molecules
4. These cells are recognized by CTLs bearing TCRs whose binding sites are complementary to the viral peptides & the infected cell is destroyed
5. A single foreign peptide presented on a cell surface is probably sufficient to invite a CTL attack
6. Since virtually all cells of body express MHC class I molecules on their surface, CTLs can combat infection regardless of the cell type affected
7. CTLs may also recognize & destroy cells that display abnormal (mutated) proteins on their surfaces; which could play a role in the elimination of potentially life-threatening tumor cells

B. Some viruses evade host the IS by suppressing MHC class I molecule expression; this makes the infected host cell effectively invisible to CTLs
1. Some metastatic tumor cells also lose MHC expression & become resistant to CTLs, but they become sensitive to attack by NK cells of the innate IS
2. NK cells bear receptors on their surfaces that recognize self MHC class I proteins on surfaces of other body cells
3. When the NK receptors bind to MHC class I proteins on a normal body cell, NK cell cytotoxic activity is inhibited
4. When an infected cell loses one or more of its MHC-I proteins, it becomes an NK cell target

C. Helper T cells recognize their Ag associated with MHC class II molecules (**MHC class II-restricted**); thus, T_H cells are activated primarily by exogenous (extracellular) Ags (bacterial cell walls or toxins)
1. MHC class II molecules are found predominantly on B cells, dendritic cells & macrophages
2. The above cells are the lymphoid cells that ingest foreign, extracellular materials & present the fragments to helper T cells
3. Helper T cells that are activated like this can then stimulate B cells to produce soluble ABs that bind to the exogenous Ag wherever it is located in body

IV. MHC role in Ag presentation does not explain the highly polymorphic nature of their genetic loci
   A. Polymorphism may give members of a population an individuality by which they can be distinguished from other members
   B. Behavioral studies in mice & humans support this - characteristic differences in body odor from person-to-person (or between mice of different strains) has been attributed to specific MHC allele differences
      1. Differences may arise from soluble MHC proteins excreted in sweat & their effects on growth of bacterial flora
      2. MHC also contributes to olfactory perception, particularly in female mammals
   C. Studies suggest that mating preferences in mice are strongly influenced by MHC genotypes & the same may be true of humans
      1. Gave women sweaty clothing worn by different men & asked them to select items with most pleasurable aroma
      2. The selected items were usually from men whose MHC loci were most different from their own
      3. Mating between individuals having different MHC alleles provides offspring with the greatest variety of different MHC molecules
      4. This would give the offspring the ability to display the widest possible range of peptides, which, in turn, would allow the recognition of widest possible range of pathogens & the best immune response

Selected Topics on the Cellular & Molecular Basis of Immunity: Distinguishing Self From Nonself

I. T cells gain their identity in the thymus
   A. When an immature T cell migrates to thymus from bone marrow, it lacks cell surface proteins that mediate T-cell function, most notably its TCRs
      1. Stem cells that will give rise to T cells proliferate in the thymus to generate a population of T cell precursors
      2. Each of these cells then undergoes the DNA rearrangements that enable it to produce a specific TCR
   B. After these DNA rearrangements, the cell is subjected to a complex screening process in the thymus
      1. This process selects for cells having potentially useful T-cell receptors
      2. Recent studies suggest that the thymus produces small quantities of a great variety of proteins normally found elsewhere in the body
      3. Production of these proteins may be under the control of a special transcription factor (called AIRE) that is present only in the thymus
      4. It is thought that the thymus recreates an environment in which developing T cells can sample proteins containing a vast array of the body's own unique epitopes
      5. T cells whose TCRs have high affinity for peptides derived from body's own proteins are destroyed; this process of negative selection greatly reduces likelihood that the IS will attack its own tissues
      6. Both types of T cells (CTLs & T_H cells) are subjected to negative selection & thus the body is protected from both humoral & cell-mediated autoimmune responses

II. When a TCR interacts with a foreign peptide on an APC surface, it must recognize both the peptide & the MHC molecule holding that peptide
   A. Thus, T cells whose TCRs do not recognize self-MHC molecules are of little value

1. The IS screens out such cells by requiring that T cells recognize self-MHC-self-peptide complexes with low affinity
2. T cells whose TCRs are unable to recognize self-MHC complexes undergo apoptosis in the thymus, a process referred to as "death by neglect"

B. In contrast, T cells whose TCRs show weak (low affinity) recognition toward self-MHC–self-peptide complexes are stimulated to stay alive, but are not activated
   1. This process of selective survival is called positive selection

III. It is estimated that <5% of thymic T cells that mature in the thymus survive these negative & positive screening events
   A. T cells become defined by a poorly understood process as either cytotoxic ($CD4^-CD8^+$) T lymphocytes or helper ($CD4^+CD8^-$) T lymphocytes
      1. Both types of T cells leave the thymus & circulate for extended periods through the blood & lymph
      2. T cells at this stage are described as naïve T cells since they have not yet encountered the specific antigen to which their TCR can bind
      3. As they pass through lymphoid tissues, naïve T cells contact various cells that either maintain their survival in a resting state or trigger their activation
   B. As they percolate through lymph nodes & other peripheral lymphoid tissues, T cells scan the surfaces of cells for the presence of an appropriate peptide bound to a self-MHC molecules
      1. $CD4^+$ cells are activated by a foreign peptide bound to a class II self-MHC molecule
      2. $CD8^+$ T cells are activated by foreign peptides bound to a class I self-MHC molecule
      3. $CD8^+$ T cells also respond strongly to cells bearing nonself-MHC molecules (transplanted organ cells from mismatched donor) – they initiate widespread attack against graft cells —> organ rejection
   C. Under normal physiological conditions, autoreactive lymphocytes (those able to react to the body's own tissues) are prevented from becoming activated by a number of poorly understood mechanisms
      1. These mechanisms operate outside of the thymus in the body's periphery
      2. A breakdown in these mechanisms leads to the production of autoantibodies & autoreactive T cells that can cause chronic tissue damage

Selected Topics on the Cellular & Molecular Basis of Immunity: Lymphocytes Are Activated By Cell Surface Signals

I. Lymphocytes communicate with other cells through an array of cell-surface proteins
   A. T cell activation requires interaction between T cell TCR & MHC-peptide complex on another cell's surface; this interaction provides specificity, which ensures that only T cells that bind Ags are activated
   B. T cell activation also requires a second signal (**costimulatory signal**)
      1. The costimulatory signal is delivered through a second type of receptor on the T cell surface
      2. This receptor is distinct & spatially separated from TCR & also not specific for a particular Ag & does not require an MHC molecule to bind
      3. The best-studied example of this interaction is the helper T cell - professional APC (DCs, macrophages) interaction

II. Activation of helper T cells by professional APCs - helper T cells recognize Ag fragments on macrophage & DC surfaces; Ag fragments are lodged in binding cleft of MHC class II molecules
   A. A costimulatory signal is delivered to a helper T cell as the result of an interaction between CD28 protein on helper T cell surface & a member of the B7 family of proteins on the APC surface
      1. The B7 protein appears on the APC surface after the phagocyte ingests a foreign Ag
   B. If the second signal is not received from the APC, the helper T cell, rather than becoming activated, will either become nonresponsive (**anergized**) or will be stimulated to undergo apoptosis (deleted)
   C. Since professional APC cells are the only ones that can deliver a costimulatory signal —> they are the only cells that can initiate a helper T cell response
      1. Thus, normal cells of the body that bear proteins capable of combining with TCRs of a T cell can not activate helper T cells
      2. Thus, the requirement for 2 signals to activate helper T cells protects normal body cells from autoimmune attack involving helper T cells

III. Prior to its APC interaction, a helper T cell is described as "resting" or G_0 cell, withdrawn from cell cycle
   A. Upon receiving the dual activation signals, a helper T cell is stimulated to reenter G_1 phase of cell cycle & eventually to progress through S phase into mitosis
      1. Interaction of T cells with a specific Ag leads to the proliferation (**clonal expansion**) of those cells capable of responding to that Ag
   B. T_H cell activation also causes it to make & secrete cytokines (most notably IL-2) along with cell division
   C. The cytokines produced by activated helper T cells act on other immune system cells (B cells, macrophages) & also act back on the helper T cells that secreted the cytokines in the first place

IV. The ultimate response of a cell in the immune system (as with non-immune system cells) is determined by a balance of positive & negative influences
   A. Interaction between CD28 & a B7 protein delivers a positive signal to a T cell leading to its activation
      1. Once the T cell has been activated, it produces another cell-surface protein called CTLA4 that is similar to CD28 in structure & also interacts with B7 proteins of the APC
      2. Unlike the CD28-B7 interaction, the CTLA4-B7 interaction leads to inhibition of T cell response
   B. The need for balance between activation & inhibition is most evident in mice that are genetically engineered to lack the gene encoding CTLA4 —> these mice die due to massive T cell overproliferation
   C. Findings with CTLA4 have been put to clinical use – patients with advanced melanoma were treated with an antibody against CTL4A in an attempt to overcome the body's state of tolerance toward tumor
      1. Of 14 patients treated with the antibody, 6 showed evidence of an autoimmune response, 3 of which exhibited regression of their tumors
      2. This preliminary study suggests that anti-CTL4A antibodies may become a useful supplement to cancer immunotherapy

V. Activation of B cells by helper T cells - helper T cells bind to B cells whose receptors bind the same Ag

A. Ag initially binds to Ig (BCR) at B cell surface & is then taken into B cell where it is processed enzymatically —> its fragments are then displayed in combination with MHC class II molecules

B. Peptide fragment recognition by TCR leads to helper T cell activation, which responds by activating B cell - B cell activation occurs after transmission of several signals from helper T cell to B cell

1. Some signals are transmitted directly from one cell surface to the other through an interaction between complementary proteins (like CD40 & CD40 ligand [CD40L])
2. CD40 - CD40L binding causes signals that help move B cell from G_0 resting state back into cell cycle
3. Other signals are transmitted by cytokines (IL-4, IL-5, IL-6, IL-10) released by T cell into the space separating it from the nearby B cell (similar to neurotransmitters across neural synapse)
   a. Interleukin-4 may stimulate B cell to switch from making IgM class ABs to IgG or IgE class
   b. Other cytokines induce proliferation, differentiation & secretory B cell activities

Selected Topics on the Cellular & Molecular Basis of Immunity: Signal Transduction Pathways Used in Lymphocyte Activation

I. Stimulation of lymphocytes occurs by mechanism similar to those used by hormones, growth factors & other chemical messengers - utilize many of the same components employed by hormones, etc.

A. When a T cell is activated by a DC or a B cell is activated by a helper T cell, a signal is transmitted from membrane to cytoplasm by tyrosine kinases, similar to those for insulin & growth factors

1. Unlike insulin receptors, lymphocyte Ag receptors lack an inherent tyrosine kinase activity
2. Instead, ligand binding to Ag receptors leads to recruitment of cytoplasmic tyrosine kinase molecules to the inner surface of the plasma membrane
3. Several different tyrosine kinases have been implicated in signal transduction during lymphocyte activation, including Src family members (e.g., Lyk & Fyn)
4. Src was the first tyrosine kinase to be identified & is the product of the first cancer-causing oncogene to be discovered

B. Activation of these various tyrosine kinases leads to a cascade of protein phosphorylation (and other events) & subsequent activation of numerous signal transduction pathways, including:

1. Activation of phospholipase C —> formation of IP_3 & DAG —> IP_3 causes marked elevation in levels of cytosolic [Ca^{2+}] & DAG causes stimulation of protein kinase C activity, respectively
2. Activation of Ras —> activation of MAP kinase cascade
3. Activation of phosphatidylinositol 3-hydroxy kinase (PI3K) —> catalyzes formation of membrane-bound lipid messengers having diverse functions in cells

C. Transmission of signals along these & other pathways & their consequent activation —> activation of a number of transcription factors (e.g., NF-kB & NFAT)

1. Activation of these transcription factors causes transcription of dozens of genes that are not expressed in resting T cells or B cells

II. One of most important responses of an activated lymphocyte is the production & secretion of cytokines, some of which may act back on the cell that released them

A. Like other extracellular signals, cytokines bind to receptors on the outer surface of target cells —> generate cytoplasmic signals that act on various intracellular targets

B. Cytokines (CKs) use a novel signal transduction pathway (the **JAK-STAT pathway**), which operates without the involvement of second messengers
   1. JAK - acronym for ***Ja***nus (2-faced Roman god who protected entrances & doorways) ***k***inases, a cytoplasmic tyrosine kinase family; its members are activated after CK binds to cell surface receptor
   2. STAT - acronym for ***s***ignal ***t***ransducers & ***a***ctivators of ***t***ranscription, a family of transcription factors that becomes activated when one of their tyrosine residues is phosphorylated by a JAK

C. Once phosphorylated, STAT molecules interact to form dimers that translocate from cytoplasm to nucleus —> they bind to specific DNA sequences, like interferon-stimulated response element (ISRE)
   1. ISREs are found in the regulatory regions of a dozen or so genes that are activated when a cell is exposed to the cytokine interferon-α (IFNα)

D. The specific response of the cell depends on the particular cytokine receptor engaged & the particular JAKs & STATs present in that cell; examples of responses mediated by JAK-STAT pathway:

1. IL-4 induces phosphorylation of the transcription factor, STAT6 (present in cytoplasm of activated B cells) —> activated STAT-6 leads to B cell Ig class switching
   2. Interferons —> induce phosphorylation of STAT1 —> induces resistance to viral infection
   3. Phosphorylation of other STATs —> progression of target cell through cell cycle

The Human Perspective: Autoimmune Diseases

Types of Autoimmune Diseases

I. The immune system requires complex & highly specific interactions between many different types of cells & molecules
   A. Numerous events take place before a humoral or cell-mediated immune response can be initiated, which makes these processes vulnerable to disruption at various stages by numerous factors
   B. Included among the various types of immune dysfunction are autoimmune diseases, which occur when the body mounts an immune response against part of itself
   C. Since T & B cell Ag receptor specificity is determined by random gene rearrangement, it is inevitable that receptors of some cell populations are directed against body's own proteins (self-antigens)
      1. Lymphocytes that bind self-antigens with high affinity tend to be removed from lymphocyte population, making the immune system tolerant toward self
      2. But some of the self-reactive lymphocytes generated in the thymus & bone marrow escape the body's negative selection processes, giving them the potential to attack normal body tissues
   D. The presence of B & T lymphocytes capable of reacting against the body's tissues is readily demonstrated in healthy individuals
      1. Isolate T cells from blood & treat them *in vitro* with normal self-protein, together with cytokine IL-2.
      2. A few cells in population are likely to proliferate to form a clone of cells that react to self-antigen
   E. Inject lab animals with purified self-antigen plus adjuvant (nonspecific substance that enhances response to injected antigen) & they mount immune response against tissues where that protein is normally found

F. Normally, B & T cells capable of reacting to self-antigens are suppressed by various mechanisms; when these mechanisms fail, a person may suffer from an autoimmune disease including:
1. Multiple sclerosis (MS)
2. Insulin-dependent diabetes mellitus (IDDM)
3. Grave's disease & thyroiditis
4. Rheumatoid arthritis
5. Systemic lupus erythematosus (SLE)

II. Multiple sclerosis (MS) – an inflammatory disease that typically strikes young adults, causing severe & often progressive neurological damage
A. MS results from an attack by immune cells and/or antibodies against the myelin sheath that surrounds nerve cell axons; these sheaths form the white matter of the central nervous system

B. The demyelination of nerves resulting from this immunologic attack interferes with the conduction of nerve impulses along axons
1. The person is left with diminished eyesight, problems with motor coordination & disturbances in sensory perception
C. A disease similar to MS (experimental allergic encephalomyelitis) can be induced in lab animals by injection of myelin basic protein, a major component of the myelin plasma membrane

III. Insulin-dependent diabetes mellitus (IDDM) – often called juvenile-onset or type I diabetes because it tends to arise in children; it results from autoimmune destruction of insulin-secreting β cells of pancreas
A. Destruction of these cells is mediated by self-reactive T cells
B. At present, patients with IDDM are administered daily doses of insulin
1. While the hormone allows them to survive, these patients are still subject to degenerative kidney, vascular & retinal disease

IV. Grave's disease & thyroiditis – autoimmune diseases of the thyroid that produce very different symptoms
A. Grave's disease – the target of immune attack is the TSH receptor on the surface of thyroid cells that normally binds the pituitary hormone thyroid-stimulating hormone (TSH)
1. In patients, autoantibodies bind to TSH receptor, causing prolonged stimulation of thyroid cells
2. This leads to hyperthyroidism (elevated blood levels of thyroid hormone)
B. Thyroiditis (Hashimoto's thyroiditis) – develops from an immune attack against one or more common proteins of thyroid cells, including thyroglobulin
1. The resulting destruction of the thyroid gland leads to hypothyroidism
2. Hypothyroidism results in decreased blood levels of thyroid hormone

V. Rheumatoid arthritis – affects ~1% of the population & is characterized by the progressive destruction of the body's joints due to a cascade of inflammatory responses
A. In a normal joint, the synovial membrane lining the synovial cavity is only a single cell layer thick
B. In persons with rheumatoid arthritis, this membrane becomes inflamed & thickened due to infiltration of autoreactive immune cells and/or autoantibodies into the joint
1. Over time, the cartilage is replaced by fibrous tissue, which causes the immobilization of the joint

VI. Systemic lupus erythematosus (SLE) – gets its name "red wolf" from the reddish rash that develops on the cheeks during the early stages of the disease
   A. Unlike other autoimmune diseases, SLE is seldom confined to a particular organ but often attacks tissues throughout the body, including the central nervous system, kidneys & heart
   B. The serum of patients with SLE contains antibodies directed against a number of components that are found in the nuclei of cells, including:
      1. Small nuclear ribonucleoproteins (snRNPs)
      2. Proteins of the centromeres of chromosomes
      3. Most notably double-stranded DNA
   C. The incidence of SLE is particularly high in women of child-bearing age, suggesting a role for female hormones in triggering the disease

Influence of Genetics and Other Factors on Autoimmune Diseases

I. Not everyone in the population is equally susceptible to developing a particular autoimmune disease
   A. Most of these disorders appear much more frequently in certain families than in general population, indicating a strong genetic component to their development
   B. Many different genes increase susceptibility to autoimmune diseases, but the genes that encode MHC class II polypeptides are most strongly linked
      1. People who inherit certain alleles of the MHC locus are particularly susceptible to developing type I diabetes (IDDM)
      2. Analysis of MHC polypeptides encoded by various HLA-DQB locus alleles is focused on one particular polypeptide chain residue (# 57), which is situated at one end of peptide-binding groove
      3. Resistance to IDDM development is correlated with the presence of an aspartic acid residue at this position, whereas susceptibility is correlated with a serine, alanine or valine at this position
      4. These amino acid differences can influence the type of peptide that the MHC molecule can bind & present to other cells
      5. MHC molecules lacking aspartic acid at position 57 can bind peptides containing negatively charged glutamic or aspartic acid residues at their C terminus
      6. On the other hand, MHC molecules containing aspartic acid at this position cannot bind these peptides
      7. It is thought that cells bearing MHC molecules encoded by susceptible allele can bind some particular peptide that stimulates formation of autoantibodies against insulin-secreting, pancreatic β cells

II. Possession of high-risk alleles may be necessary for an individual to develop certain autoimmune diseases, but it is not the only contributing factor
   A. Studies of monozygotic (genetically identical) twins indicate that if one twin develops an autoimmune disease, the likelihood that the other twin will also develop the disease ranges from ~25 – 75%
      1. If genetics were the only contributing factor, the other twin should get the disease 100% of the time
      2. Studies such as these demonstrate that environmental factors play a role
   B. The importance of pathogens in the development of autoimmune disorders was first demonstrated in studies of rheumatic fever
      1. This condition can develop in children several weeks after a streptococcal infection of the throat (strep throat)

2. Rheumatic fever develops when heart tissue is attacked by antibodies produced in response to the streptococcal bacteria
3. Heart tissue becomes a target of these antibodies due to a phenomenon called molecular mimicry
4. A component of the bacterial cell wall is similar to that of a glycoprotein on the surface of cells lining the heart valves
5. Thus, antibodies produced in response to the bacterial infection can cross-react with heart tissue

C. In the case of multiple sclerosis, it is found that respiratory or intestinal viral infections can activate self-reactive T cells & trigger relapses in patients who have gone into remission
1. Some think viral infection is underlying cause of MS; this is supported by reports of MS epidemics

D. Studies on animals have also indicated the importance of pathogens
1. Mice that lack a gene for the cytokine IL-2 (IL-2 knockout mice) develop an inflammatory bowel disorder resembling human ulcerative colitis
2. This condition only develops in these lab animals if they are exposed to infectious agents of the normal environment; if they are raised under germ-free conditions, they remain disease-free

Promising New Treatments for Autoimmune Diseases

I. There are a number of promising new treatments for autoimmune diseases – tested in animal models (animals that can be made to develop diseases similar to those of humans); clinical trials are under way
A. Treatment with immunosuppressive drugs that block the autoimmune response
B. Induction of a return to a tolerant state to specific antigens so that the body stops producing autoantibodies & autoreactive T cells
C. Interference with the autoimmune response by administering antibodies
D. Blocking the effect of inflammatory cytokines by administration of suppressive cytokines
E. Blocking body's ability to mount immune response by immunization

II. Treatment with immunosuppressive drugs (cyclosporin A) that block the autoimmune response
A. These drugs are nonspecific & inhibit all types of immune responses & render the patient highly susceptible to dangerous infections

III. Induction of a return to a tolerant state to specific antigens so that the body stops producing autoantibodies & autoreactive T cells
A. Administration of altered peptide ligands (APLs) - thought to bind to TCRs in a suboptimal manner, blocking T-cell activation & reducing secretion of inflammatory cytokines (TNF-α & IFN-γ)
B. The drug Copaxone has been shown to reduce the frequency of relapses in patients with MS; it is a synthetic peptide whose structure resembles that of myelin basic protein
C. APLs have been reported to elicit severe allergic side-effects, which has raised some questions about their use

IV. Interference with the autoimmune response by administering antibodies
A. An AB directed against CD3 protein on T-cell surface is being tested in type I diabetes treatment; it inactivates or destroys cells that attack pancreatic β cells; may lead to widespread immune deficiency

B. Antibodies against the cytokine TNF-α (Remicade) have been approved for treatment of rheumatoid arthritis; they can have dramatic curative effects in some patients
C. An antibody directed against the integrin subunit α4 appears to block the recruitment of white blood cells to inflammation sites; effective in clinical trials on patients with Crohn's disease & MS
D. Rituximab – monoclonal AB used to treat B cell lymphoma (causes B cell depletion); induced B cell deficiency appears not to limit patients' ability to mount immune responses against infectious agents

V. Blocking the effect of inflammatory cytokines by administration of suppressive cytokines – IL-4 has shown promise in treatment of IDDM & IFN-β1a (Avonex) has been approved for MS treatment

VI. Blocking body's ability to mount immune response by immunization – trials have been carried out on MS patients who have been immunized with TCR component that recognizes myelin basic protein self-Ag
A. Body responds to immunization by making antibodies that attack those specific T cells that are responsible for the disease

Experimental Pathways: The Role of the Major Histocompatibility Complex in Antigen Presentation

I. Hugh McDevitt et al. (Scripps Found. & Stanford, 1973) – showed that susceptibility of mice to a particular pathogen depends on allele found at one of the MHC loci; used lymphocytic choriomeningitis virus (LCMV)
A. Found LCMV causes lethal brain infection in mice that are homozygous or heterozygous for H-2^q allele, but get no infections in mice homozygous for H-2^k allele at this locus

II. Rolf Zinkernagel & Peter Doherty (Australian Nat'l Univ.) – examined the role of cytotoxic T lymphocytes (CTLs) in the development of this disease
A. Planned experiments to correlate CTL activity levels with disease severity in mice having different MHC genotypes (haplotypes); monitored CTL activity as follows:
1. Fibroblast (FB; L cell) monolayers from 1 mouse were grown in culture & then infected with LCMV
2. Infected FBs were then overlaid by spleen cell prep from mouse infected with LCMV 7 days earlier (IS had time to make CTLs against virus-infected cells); CTLs concentrated in infected animal's spleen
3. To monitor effectiveness of CTL attack on cultured L cells, L cells were first labeled with chromium (^{51}Cr) radioisotope
4. Chromium is used as a marker for cell viability; as long as cell stays alive, ^{51}Cr stays inside cell; if CTL lyses a cell during experiment, ^{51}Cr is released into the medium
B. Found that the level of CTL activity against the cultured FBs, as measured by ^{51}Cr release, depended on the relative genotypes of the FBs & spleen cells
1. The FBs used in the experiment were from an inbred strain of mice homozygous for the allele H-2^k at the H-2 locus
2. When spleen cells were prepared from mice having an H-2^k allele (e.g., CBA/H, AKR & C3H/HeJ strains of mice), the L cells were effectively lysed
3. Spleen cells taken from mice bearing H-2^b or H-2^d alleles at this locus were unable to lyse the infected FBs; the ^{51}Cr released is approximately the same when noninfected FBs are used in the assay

C. Next they tested LCMV-activated spleen cells from H-2^b mice against various types of infected cells
   1. CTLs would only lyse infected cells having the same H-2 genotype, in this case H-2^b
   2. These studies were the first evidence that MHC molecules on the surface of an infected cell restricts its interactions with T cells

III. These & other experiments during 1970s raised questions about MHC protein role in immune cell function; another line of study focused on mechanism by which T cells were stimulated by particular Ags
   A. Studies showed that T cells respond to Ag bound to surface of other cells; it was presumed that Ag being displayed had simply bound to antigen-presenting cell (APC) surface from surrounding medium
   B. Alan Rosenthal (NIH) & Emil Unanue (Harvard, mid-1970s & early 1980s) – showed that Ag had to be internalized by APC & subjected to some processing before it could stimulate T-cell proliferation
      1. Most of these studies were done in cell culture utilizing T cells activated by macrophages that had been previously exposed to bacteria, viruses or other foreign material
      2. Can distinguish Ag simply bound to APC surface from Ag processed by metabolic activity
      3. This is done by comparing events occurring at low temperatures (4°C; at which metabolic processes are blocked) with those occurring at normal body temperatures
      4. Macrophages were incubated with Ag for 1 hr at either 4°C or 37°C & then tested for their ability to stimulate T cells prepared from lymph nodes
      5. T-cell stimulation was measured by incorporation of [^{3}H]thymidine into DNA that accompanies cell proliferation
      6. At lower Ag concentrations, macrophages were nearly 10 times more effective at stimulating T cells at 37°C than at 4°C, suggesting that Ag processing requires active metabolic events
      7. Treatment of cells with sodium azide, a cytochrome oxidase inhibitor, also inhibited the appearance of Ag on T-cell surfaces, indicating that Ag presentation requires metabolic energy
   C. Later experiments by Kirk Ziegler & Emil Unanue showed that sequestration occurred as extracellular antigens were taken into macrophages by endocytosis & delivered to cell's lysosomal compartment
      1. To determine if lysosomes are involved in a particular process, the cells were treated with substances, like ammonium chloride or chloroquine that disrupt lysosomal enzyme activity
      2. Both of these agents raise the lysosomal compartment pH, which inactivates the acid hydrolases
      3. The studies employed *Listeria monocytogenes* bacteria & tested the effect of the above chemicals on processing & presentation of antigen derived from *Listeria*
      4. Neither of these substances affected antigen uptake (endocytosis), but both of them markedly inhibited antigen processing & its ability to stimulate binding of T cells to the macrophage
      5. These data were among the first to suggest that extracellular antigen fragmentation by lysosomal proteases may be an essential step in the preparation of extracellular antigens prior to presentation

IV. Other studies continued to implicate MHC molecules in the APC – T cell interaction
   A. Ziegler & Unanue treated macrophages with ABs directed against MHC proteins encoded by H-2 locus
      1. They found that these antibodies had no effect on the uptake or catabolism of antigen, but markedly inhibited the macrophages from interacting with T cells

2. Inhibition of T cell binding to macrophages was obtained even when macrophages were exposed to the antibodies before addition of antigen

B. Evidence from this & other studies showed that interaction between a T cell & a macrophage depended on recognition of 2 components on APC surface: Ag fragment being displayed & an MHC molecule

C. 2 models of antigen recognition by the T cell were considered likely possibilities
   1. T cells possess 2 distinct receptors, one for antigen & another for the MHC protein
   2. A single T-cell receptor recognizes both the MHC protein & the antigen peptide on the APC surface simultaneously

D. The balance of opinion began to shift in favor of the one-receptor model as evidence began to point to a physical association between MHC proteins & displayed antigens
   1. It was shown that antigen that had been processed by T cells could be isolated as a complex with MHC proteins
   2. Cultured T cells from H-2^k mice were incubated with radioactively labeled antigen for 40 minutes
   3. After the incubation, processed antigen was prepared from the cells & passed through a column containing beads coated with antibodies directed against MHC proteins
   4. If beads were coated with ABs against H-2^k protein, an MHC molecule present in the T cells, lots of radioactive Ag stuck to beads, indicating the association of processed Ag with the MHC protein
   5. If beads were instead coated with antibodies against H-2^b protein, an MHC protein not present in the T cells, relatively little radioactive antigen remained in the column

V. The atomic structure of molecules involved in T-cell interactions – studies over the past decade or so have examined MHC class I molecules of the type found on the surfaces of virally-infected cells

A. Don Wiley et al. (Harvard, 1987) – published the first 3D portrait of an MHC molecule based on X-ray crystallographic studies; they found that MHC molecules consist of:
   1. A heavy chain with 3 extracellular domains (α_1, α_2 & α_3) & a single membrane-spanning segment **and**
   2. An invariant β_2m polypeptide

B. They examined the structure of the extracellular (soluble) portion of the MHC molecule (α_1, α_2, α_3 & β_2m) after removing the transmembrane anchor
   1. The outer (antigen-bearing) portion of the protein is constructed from the α_1 & α_2 domains; the inner surfaces of these domains form the walls of a deep groove ~25 Å long & 10 Å wide
   2. This groove acts as the binding site for peptides produced by antigen processing in the cytoplasm
   3. The sides of the antigen-binding pocket are lined by α-helices from the α_1 & α_2 domains & the bottom of the pocket is lined by β-sheet that extends from these same domains across the midline
   4. The helices are thought to form relatively flexible side-walls enabling peptides of different sequence to bind within the groove

C. Later X-ray crystallographic studies described the manner in which peptides are positioned within the MHC-antigen binding pocket
   1. The spatial arrangement of several naturally processed peptides situated within the antigen-binding pocket of a single MHC class I molecule (HLA-B27) was determined
   2. The backbones of all the peptides bound to HLA-B27 share a single, extended conformation running the length of the binding cleft

3. The N- & C-termini of peptides are precisely positioned by numerous H bonds at both ends of cleft
4. The H bonds link the peptide to a number of conserved residues in the MHC molecule that are part of the sides & bottom of the binding groove

D. Ian Wilson et al. (Scripps Research Inst., La Jolla CA) – reported on X-ray crystallographic structure of a mouse MHC class I protein complexed with 2 peptides of different length
1. The overall structure of the mouse MHC protein is similar to that of the human MHC protein
2. In both MHC proteins, peptides are bound in an extended conformation deep within the MHC molecule binding groove
3. This extended conformation allows for numerous interactions between the side chains of the MHC molecule & the backbone of the bound peptide
4. Since the MHC interacts mostly with a peptide's backbone rather than its side chains, there are very few restrictions on the particular amino acid residues that can be present at various binding pocket sites
5. As a result, each MHC molecule can bind a diverse array of antigenic peptides

VI. An MHC-peptide complex projecting from infected cell's surface is only half of the story of immunologic recognition; the other half is represented by T-cell receptor (TCR) projecting from the cytotoxic T cell

A. A TCR can somehow recognize both an MHC & its contained peptide, but how this happens has eluded researchers due to difficulties in preparing TCR protein crystals suitable for X-ray crystallography

B. These difficulties were eventually overcome & both the Wiley & Wilson labs (1996) provided a 3D portrait of the interaction between an MHC-peptide & a TCR
1. MHC class I molecule has extended peptide antigen embedded within the protein's binding pocket
2. A TCR consists of α & β polypeptide chains, each chain with a variable (V) & a constant (C) portion
3. Like immunoglobulins, the variable portion of each TCR subunit contains regions that are exceptionally variable (hypervariable)
4. Hypervariable regions form protruding loops that fit snugly over MHC-peptide complex outer end & are called complementarity-determining regions (CDRs) since they determine TCR binding properties
5. The CDRs of the TCR interact with the α-helices of the α_1 & α_2 domains of the MHC, as well as exposed residues of the bound peptide
6. Central TCR CDRs with the greatest sequence variability interact mostly with centrally situated bound peptide, whereas outer CDRs with a less variable sequence interact most closely with MHC α-helices

C. Because of these interactions, the TCR meets both of its recognition responsibilities
1. It recognizes the bound peptide as a foreign antigen
2. It recognizes the MHC as a self-protein

LECTURE HINTS

An Overview of the Immune Response

Quite often, a brief overview of the immune system is the most I have time for. I often incorporate discussions of immune system cells into other lectures, such as the signal transduction, the cell surface and even the techniques lectures with respect to fluorescence microscopy and the use of

antibodies in research. Describe the cells involved in the immune response and define their functions at least briefly. Take more time, if you have it, to go into more detail. My guess is that, under most circumstances, this would not be necessary. Describe the weapons that the immune system uses to remove pathogenic cells and viruses from the body. Differentiate between the innate immune responses that the body can immediately mount against an invading pathogen and the acquired immune responses that require a lag period before they can be brought up to speed. Point out that the innate response is a more general one, that it does not change with the nature of the invading pathogen and that it is considered to be the body's first line of defense. On the other hand, the acquired response, while slower, is a much more specific one.
Distinguish between the two broad categories of acquired immune responses: humoral immunity and cell-mediated immunity. Explain that humoral immunity involves the production of specific antibodies directed against foreign materials (pathogens, toxins, etc.) situated outside the body's cells, while cell-mediated immunity involves the action of T cells and other cells that aid the body in mounting an immune response against an infected or foreign cell (and maybe a potential cancer cell). Cell-mediated immunity is, of course, needed since antibodies cannot attack foreign materials inside cells, while cell-mediated immune responses can. Point out that while there is overlap between the two kinds of immunity, they can be dissociated to a large extent. Mention the example of congenital agammaglobulinemia to illustrate this fact.

The Clonal Selection Theory

Describe the differences between the first explanation for the ability of immune cells to mount such a specific response and the presently accepted explanation. It was originally thought that the antigens found on foreign material instructed immune cells how to construct an appropriate and complementary antibody. It has since been convincingly demonstrated that the antigens found on foreign material select those immune cells capable of interacting with that antigen and cause them to proliferate. Next, these proliferated cells will either secrete the appropriate antibody that can interact with the antigen or if they are T cells, they will develop the ability to carry out cell-mediated immunity against cells bearing the appropriate antigen. This latter proposal has come to be called the clonal selection theory.

If you have time, describe the experiment that suggests the correctness of the clonal selection theory, an experiment in which mouse spleen cells are passed through a column coated with antigen. See the text for the details of this experiment. Outline the major steps in the proposed process of clonal selection. Emphasize the importance of the cells in the clone that respond immediately to the stimulation by antigen, as well as the significance of the memory cells that linger in the body until the same antigen is encountered once more (immunization). Also, address the issue of immune tolerance and broach the topic of autoimmune diseases. This usually grabs the attention of students because they frequently wish to relate what they are learning to more practical issues, like the implications of these principles for human health. Discuss lupus erythematosus, multiple sclerosis, rheumatoid arthritis and other diseases thought to be examples of autoimmunity.

Discuss smallpox and tetanus vaccinations, the principles underlying them and the requirement for booster shots, in some cases. The story of Jenner's discovery of vaccination and his risky experiment is a good one. Relate it to what is known now about the development of immunity. Integration of the history with the more elaborate body of knowledge we now have is, I believe, an effective teaching strategy. Mention the concept of passive immunity as well.

T Lymphocytes: Activation and Mechanism of Action

T cells are activated by clonal selection in a fashion similar to the process of B cell activation. Like B cells, T cells are activated by a specific antigen; however, the antigen must be bound to the surface of an antigen-presenting cell (APC). Furthermore, the whole antigen is not what activates the T cell; rather, it is fragments of the antigen presented on the surface of an APC that activates the T cells. Point out that almost any cell can act as an APC and that some cells act as professional APCs, like macrophages and dendritic cells. Further emphasize that macrophages blur the lines between innate and acquired immunity, since it is their action in innate immunity that provides them with the opportunity to acquire antigens to present on their surface, thus initiating an acquired immunity pathway.

Explain that T cells, once activated, produce cytokines, which are small proteins secreted from the T cells that bind to receptors on a target cell and thus initiate an intracellular signal. The signal generated by the cytokine's binding alters cell activity, causing it to divide, differentiate or secrete its own cytokines. Describe the two major categories of T cells: cytotoxic T cells and helper T cells. Outline to whatever depth you feel advisable the roles these cells play in the immune response.

Antibody Structure

Describe the structure of antibodies and the different classes of immunoglobulins. Describe the two different classes of light chains (kappa [κ] and lambda [λ]) and the heavy chains. Emphasize the structure of the best-known immunoglobulin, IgG, with its two identical heavy chains and two identical light chains in a Y-shaped arrangement. Outline what is known about the functions and structures of the different antibody classes.

Point out that both heavy and light chains are composed of variable and constant portions. The variable portions of both heavy and light chains appear to be largely responsible for the ability of antibodies to bind to so many different antigens with such high affinity, especially those regions called the hypervariable regions that, as the name suggests, are especially variable. These hypervariable regions are clustered at the ends of each arm of the antibodies and play a significant role in forming the antigen-combining site. The constant portions of the heavy chain of antibodies account for the differences in function of the different antibody classes. Describe what is known about the functions of the different classes of antibodies.

Generation of Antibody Variability By DNA Rearrangement

Describe the rearrangement of DNA that leads to the varying associations of different variable and constant portions to form a complete light chain. Use kappa light chains as an example. Describe the Tonegawa experiment that provided supporting evidence in favor of DNA rearrangement. Delineate the process whereby one of the stretches of DNA coding for a V_K gene is attached to one of the J_K DNA segments to form a full kappa V gene.

Explain how DNA rearrangement is largely responsible for the ability of a relatively small number of genes to generate the staggering number of different antibodies that the human immune system can produce (>2,000 light chains and ~100,000 heavy chains and >200 million different species of antibodies from a few hundred genetic elements in the germ line). Explain how you got the numbers mentioned above to make sure that your students fully understand the concept. Point out that further variability can be obtained after DNA rearrangement through

somatic hypermutation in which sites in the genes are altered. The rearranged DNA encoding the V region has a mutation rate 10^5 times greater than that of other genetic loci in the same cell.

The Major Histocompatibility Antigens

Describe the importance of the proteins of the major histocompatibility complex to tissue transplantation and the role they play with respect to the presentation of antigens on the surface of antigen-presenting cells. Define the roles of the MHC class I (displaying antigens that originate in the cell cytoplasm) and MHC class II molecules (displaying antigen fragments taken into the cell via endocytosis) in the immune response. If time permits, point out the differences between the processing of MHC class I and II proteins. Explain the interaction of MHC class I molecules with cytotoxic T cells and the interaction of MHC class II molecules with helper T cells. Point out the ability of some viruses to evade the immune system by suppressing the synthesis of MHC class I molecules. Ask the students how this would allow said evasion. If there is no MHC class I expression, the cells will be for all intents and purposes invisible to cytotoxic T cells. The same is true of some metastatic tumor cells. Perhaps the most interesting aspect of the MHC story is the speculation about the natural purpose of the MHC complex. Obviously, tissue transplant, which is how the MHC complex was first discovered and comprises its best known function nowadays, is not the natural function of these molecules. It has been speculated that they have some effect on body odor and may help to ensure that mating occurs between individuals with different MHC alleles. A wider range of MHC alleles may make an organism better able to recognize the widest range of pathogens. This is the kind of story that can hold student interest since mating is something most of them have some interest in. You can probably wring some humor out of such a story as well.

Distinguishing Self From Nonself

This is an extremely important concept for the students to understand. Mention it a couple of times throughout the lecture series. Explain that early in the development of the immune system, cells that could respond to antigens present in the organism are eliminated. Theoretically, this leaves behind only immune system cells that can respond to the presence of foreign antigens. Also, emphasize the importance of the costimulatory signal that must be received from a professional antigen-presenting cell to allow the helper T cell to respond and initiate an immune response. Since these cells can generally only present foreign, exogenous antigens on their surfaces, the antigens of normal body cells are not presented. Thus, the organism normally raises no immune response against antigenic determinants present within its body, while still maintaining the ability to respond to foreign antigens. Explain how graft-versus-host reactions can arise and relate this to the way tissue transplants work.

Activation of Lymphocytes by Cell Surface Signals

Point out to the students that the binding of antigens to the surface of a cell of the immune system is a lot like the binding of a hormone or growth factor to a cell surface receptor. As with hormones, the binding at the cell surface trips off a series of reaction cascades of protein phosphorylation and activation of at least three distinct signal transduction pathways: phospholipase C, Ras, and phosphatidylinositol 3-hydroxy kinase. This will give the students another opportunity to see the

wide distribution of such pathways in cell physiology. Finish up with a brief discourse on the JAK-STAT pathway.