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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOMEDICAL ENGINEERING

UNIT – I -Introduction to Immunology – SBMA1302

IMMUNE SYSTEM

The **body's defense** against:

- disease causing organisms or infectious agents
- malfunctioning cells or abnormal body cells as cancer
- foreign cells or particles

Basic Immunity

Depends on the ability of the immune system to distinguish between ***self*** and ***non-self-*** molecules

- ***Self*** molecules are those components of an organism's body that can be distinguished from foreign substances by the immune system
  - ***Autoimmunity*** is an immune reaction against self molecules (causes various diseases)
- ***Non-self*** molecules are those recognized as foreign molecules
  - One class of non-self molecules are called **antigens** (short for *anti*body *gen*erators) and are defined as substances that bind to specific immune receptors and elicit an immune response
  - Immune System Components:
  - **specific cells** - lymphocytes, macrophages, etc., originate from precursor cells in the bone marrow and patrol tissues by circulating in either the blood or lymphatics, migrating into connective tissue or collecting in immune organs

lymphatic organs- thymus, spleen, tonsils, lymph nodes

diffuse lymphatic tissue -collections of lymphocytes and other immune cells dispersed in the lining of the digestive and respiratory tracts and in the skin.

History of Immunology

The word "immunity" (L: *immunis* - free of) was used in the context of being free of the burden of taxes or military conscription. The history of immunology is really slightly more than 100 years if you consider Louis Pasteur as the "Father of immunology" as most do. If one thinks about cellular immunology, the real history begins in the late 1950s.

From early writings, it is clear that primitive man knew about disease and its ravages. One finds in the Babylonian Epic of Gilgamesh (2000 B.C. - Mesopotamian hero) records of the presence of pestilence and disease. In other, more recent writings from old dynasties of ancient Egypt, one finds even more descriptions of disease. Further, one can even identify the disease of which they spoke. Recall that, in those days, disease and pestilence was punishment rendering as a result of bad deeds or evil thoughts. Even the old testament is filled with pestilence that God wrought upon those who crossed him. From these writings, it

is equally apparent that man knew that once he had been afflicted with disease, if he survived, he was normally not able to contract it again.

The science of immunology grew from the common knowledge that those who survived many of the then common infectious diseases, rarely contracted that disease again. This was an observation that was made long before the establishment of the germ theory of disease. Louis Pasteur and Robert Koch were instrumental in defining microorganisms as the etiological agents of a large number of diseases.

In 430 B.C., Thucydides recorded that while the plaque was raging in Athens, the sick and dying would have received no attention had it not been for those individuals who had already contracted the disease and recovered and recognized their immune status. Beginning around 1000 A.D., the ancient Chinese practiced a form of immunization by inhaling dried powders derived from the crusts of smallpox lesions. Around the fifteenth century, a practice of applying powdered smallpox "crusts" and inserting them with a pin or poking device into the skin became commonplace. The process was referred to as **variolation** and became quite common in the Middle East. However, the primary intent of variolation was that of preserving the beauty of their daughters and no mention was made of saving lives.

From Turkey, the process of variolation can be traced to the inhabitants of a country called Arcassia. The people that populated this land were poor but were blessed with large numberof beautiful women, which unfortunately was the chief trade and very important to the Arcassian economy. Most sales were to the Sultan of Turkey.

Eventually, the process was popularized in Great Britain, largely through the efforts of Mary Wortley Montagu. It was vehemently opposed by the Church and was highly discouraged, particularly if one were Christian. The clergy stated that it could only work of an "Un Christian" who was an infidel in the eyes of the Lord.

To complicate matters more, because there was no standardization of the inoculum engrafted, the practice occasionally resulted in death/disfigurement from smallpox. This coupled with the widespread acceptance of herbal medicine resulted in it not being widely accepted. In 1721, Mary Wortley Montagu’s daughter was the first person to be engrafted in Great Britain

The first step to a safer procedure was to substitute material derived from the lesion of a cowpox (vaccinia) for the inoculation. Cowpox is a benign disease due to infection with a virus closely related to the smallpox (variola) virus.Some notable events in "early" Immunology

1774- Benjamin Jesty, a farmer who inoculated his wife with the vaccinia virus obtained from farmer Elford of Chittenhall, near Yetminster. First record of anyone using vaccinia

virus To further advance the fledgling science of immunology required the development of the **Germ theory of disease.** It is here that Louis Pasteur played a pivotal role in the evolution of the science. While Pasteur's work at the Pasteur Institute in Paris was concerned with bacterial infectious disease, he was most concerned with the *prevention* of diseases that bacteria caused and how the human body was changed subsequent to infection so as to resist further insults. **Louis Pasteur**became the first experimental immunologist.to "protect" against smallpox.

In 1798, Jenner inoculated a young by named James Phipps with material obtained from a cowpox lesion. The results were conclusive but were met with great resistance by the Church. Ignorance can be hard to combat!! For this feat, Jenner received a cash prize of 30,000 pounds . The acceptance of Jenner's thesis was strengthened when 70 of the principal physicians and surgeons of London threw their weight behind him. He was elected to membership to all of the learned societies throughout Europe with the **exception** of the College of Physicians. They required that he pass an examination in Classics which Jenner refused.

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First quest was with the disease chicken cholera. It was known that chicken cholera was due to infection with the "chicken cholera bacillus". Pasteur had a flask of the organism which he inadvertently left on the bench over the summer (I guess he took "summer breaks"). When Pasteur again turned his attention to the organism, he found that the culture had lost its ability to cause disease in the chicken. Briefly, this is what Pasteur did. Desiring to infect several chickens, he took his "old but viable" culture of chicken cholera bacillus and injected 8 chickens with it. Surprising, the chickens did NOT die in the usual period of time. In fact, they did not even get sick! Somewhat vexed by the loss of time, he prepared a new stock of bacteria and re-injected the original 8 AND 10 new fresh chickens (he had to make up for lost time). After 48 hours, the 10 newly injected chickens were "sick and dying" while the original 8 were the "picture of health". Pasteur envisioned that somehow the original 8 chickens had been "changed" by the old culture and were no longer susceptible (he tried three more times to kill those stupid 8 chickens but they never got sick).

As a result of his work, Pasteur said that the virulent chicken cholera bacillus had become **attenuated** by sitting on the bench over the summer months. The similarity between this situation and Jenner's variolation with the vaccinia virus was immediately apparent to him in honor of Jenner called his treatment vaccination.

In 1886, Theobold Smith (an American microbiologist) demonstrated that *heat killed* cultures of chicken cholera bacillus were also effective in protection from cholera. This demonstrated that the microorganisms **did not have to be viable** to induce the protection.

Pasteur proceeded to do the same with anthrax. Robert Koch had shown that the disease was due to a bacterium called *Bacillus anthracis*. He maintained the culture under adverse laboratory conditions (incubation at 42-43C) and in this manner, he produced the vaccine that was used for the famous demonstration at Pouilly-le-Fort.

On May 5, 1881 Pasteur vaccinated 24 sheep, 1 goat, and 6 cows with five drops of the living attenuated anthrax bacillus. On May 17, he inoculated all of the animals with a less attenuated strain. On May 31, all of the animals received **viable virulent** anthrax bacilli. Additionally, 24 more sheet, 1 goat and 3 cows received the virulent microorganism without the protection of the vaccination. On June 2, 1881 all of the non vaccinated animals had died while only two sheep of the protected group had succumbed. One of the sheep had died due to pregnancy complications.

A third means of virulence attenuation was soon found to involve the passage of the microorganism in an unnatural host. The most dramatic demonstration of a vaccine's effectiveness was with rabies. Isolating the virus from a fox, Pasteur passed the virus in an "unnatural host" the rabbit. By infecting one rabbit, allowing it to become ill, and then re-isolating the virus and injecting a new rabbit, Pasteur "selected" for variants of the virus that were less pathogenic for the fox. Pasteur dried the spinal cord taken from an infected rabbit and prepared a vaccine from it. To test it however, he needed a human subject who was undoubtedly going to come down with rabies.

The first human trial was on July 6, 1885. A nine-year old lad named Joseph Meister had been severely bitten by a rabid dog two days earlier. His parents knew that he "was a goner" and were desperate for any ray of hope. They heard of Pasteur's work and traveled to Paris, France in hopes that Pasteur would "work a miracle". Pasteur injected the attenuated virus into young Joey and they waited. Well, you can guess the rest of the story. Joseph Meister, after receiving the immunization, survived rabies. This was the first known case of an individual being bit and surviving rabies. Joseph Meister was later to become the gate porter of the

Pasteur Institute in Paris and served as guard to Pasteur's crypt. Within a year, over 350 people bitten by rabid animals had been treated with no fatalities.

In 1888, Roux and Yersin discovered the diphtheria toxin. Two years later, in 1890, Von Behring and Kitasato demonstrated the presence of **anti-toxin** in the blood of individuals recovering from diphtheria. Von Behring was the first to use this antiserum in treating active disease. Forerunner to what we call 'serotherapy' today.

The ideas of circulating neutralizing anti-toxins predominated early immunological thought and the French and German schools dominated immunological research. At the turn of the century, immunology developed into two schools of thought.

1. Humoralists - immunity was due to *humoral* substances, i.e. antibodies. A Pioneer in this area was Paul Ehrlich. He proposed what was the most plausible humoral theory of antibody formation the 'side chain theory'. Emil Von Behring (worked at the Koch Institute in Germany) used serum to treat diseases. Germans were big proponents of **humoral immunity**.
2. Cellularists - immunity due to the existence of 'phagocytic' cells within our bodies. The pioneer here was Eli Metchnikoff and he became the strongest proponent of **cellular immunity** after observing water Daphnia phagocytose smaller materials and examining blood cells devour foreign bacteria in blood samples. Metchnikoff was allied with Louis Pasteur (he worked at the Pasteur Institute) and he had many a vitriolic fights with the Germans who were proponents of humoral immunity.

Today, we know that immunity is due to both of these facets. We will address humoral antibody in the form of immunoglobulins and we will talk about Cellularists when we deal with T cell biology and cytotoxicity with regulation.

1903, Maurice Arthus, described the localizing allergic reaction called the Arthus response. In 1905, a Frenchman by the name of Von Pirquet shocked the world when he provided evidence that immune responses can be deleterious. He was studying serum sickness, a form of hypersensitivity or allergy.

1930, American scientists Landsteiner and Kabat described detailed experiments of the specificity of the immune response by chemically altering antigens. In 1944-45, another American named Peter Medawar provided the immunological basis of transplantation immunology.

1958, clonal selection theory as proposed by Sir McFarland Burnet and Neils Jerne

1960, Porter and Edelman enzymatically digested antibodies and we learned about their chemical structure.

1960, The first real demonstration of a cellular basis for humoral and cellular immunity. The terms T and B cells became vernacular.

There is, of course, a lot more immunology. The late 60's to early 70's have been referred to as the beginning of modern immunology. The molecular/genetic techniques of the 70's have revolutionized our understanding of how the immune system works.

Timeline of immunology:

- 1549 - The earliest account of inoculation of smallpox (variolation) occurs in Wan Quan's (1499– 1582) *Douzhen Xinfa*
- 1718 –smallpox vaccination in Ottoman Empire realized by west. Lady Mary Wortley Montagu, the wife of the British ambassador to Constantinople, observed the positive effects of variolation on the native population and had the technique performed on her own children.
- 1796 – First demonstration of vaccination smallpox vaccination (Edward Jenner)
- 1837 – Description of the role of microbes in putrefaction and fermentation (Theodore Schwann)
- 1838 – Confirmation of the role of yeast in fermentation of sugar to alcohol (Charles Cagniard-Latour)
- 1840 – Proposal of the germ theory of disease (Jakob Henle)
- 1850 – Demonstration of the contagious nature of puerperal fever (childbed fever) (Ignaz Semmelweis)
- 1857-1870 – Confirmation of the role of microbes in fermentation (Louis Pasteur)
- 1862 – phagocytosis (Ernst Haeckel)
- 1867 – Aseptic practice in surgery using carbolic acid (Joseph Lister)
- 1876 – Demonstration that microbes can cause disease-anthrax (Robert Koch)
- 1877 – Mast cells (Paul Ehrlich)
- 1878 – Confirmation and popularization of the germ theory of disease (Louis Pasteur)
- 1880 – 1881 -Theory that bacterial virulence could be attenuated by culture

in vitro and used as vaccines. Proposed that live attenuated microbes produced immunity by depleting host of vital trace nutrients. Used to make chicken cholera and anthrax "vaccines" (Louis Pasteur)

- 1883 – 1905 – Cellular theory of immunity via phagocytosis by macrophages and microphages (polymorhonuclear leukocytes) (Elie Metchnikoff)
- 1885 – Introduction of concept of a "therapeutic vaccination". Report of a live "attenuated" vaccine for rabies (Louis Pasteur).
- 1888 – Identification of bacterial toxins (diphtheria bacillus) (Pierre Roux and Alexandre Yersin)
- 1888 – Bactericidal action of blood (George Nuttall)
- 1890 – Demonstration of antibody activity against diphtheria and tetanus toxins. Beginning of humoral theory of immunity. (Emil von Behring) and (Kitasato Shibasaburō)
- 1891 – Demonstration of cutaneous (delayed type) hypersensitivity (Robert Koch)
- 1893 – Use of live bacteria and bacterial lysates to treat tumors- "Coley's Toxins" (William B. Coley)
- 1894 – Bacteriolysis (Richard Pfeiffer)
- 1896 – An antibacterial, heat-labile serum component (complement) is described (Jules Bordet)
- 1900 – Antibody formation theory (Paul Ehrlich)
- 1901 – blood groups (Karl Landsteiner)
- 1902 – Immediate hypersensitivity anaphylaxis (Paul Portier) and (Charles Richet)
- 1903 – Intermediate hypersensitivity, the "Arthus reaction" (Maurice Arthus)
- 1903 – Opsonization
- 1905 – "Serum sickness" allergy (Clemens von Pirquet and (Bela Schick)
- 1909 – Paul Ehrlich proposes "immune surveillance" hypothesis of tumor recognition and eradication
- 1911 – 2nd demonstration of filterable agent that caused tumors (Peyton Rous)
- 1917 – hapten (Karl Landsteiner)
- 1921 – Cutaneous allergic reactions (Otto Prausnitz and Heinz Küstner)
- 1924 – Reticuloendothelial system
- 1938 – Antigen-Antibody binding hypothesis (John Marrack)

- 1940 – Identification of the Rh antigens (Karl Landsteiner and Alexander Weiner)
- 1942 – Anaphylaxis (Karl Landsteiner and Merill Chase)
- 1942 – Adjuvants (Jules Freund and Katherine McDermott)
- 1944 – hypothesis of allograft rejection
- 1945 – Coombs Test aka antiglobulin test (AGT)
- 1946 – identification of mouse MHC (H2) by George Snell and Peter A. Gorer
- 1948 – antibody production in plasma B cells
- 1949 – growth of polio virus in tissue culture, neutralization with immune sera, and demonstration of attenuation of neurovirulence with repetitive passage (John Enders) and (Thomas Weller) and (Frederick Robbins)
- 1951 – vaccine against yellow fever
- 1953 – Graft-versus-host disease
- 1953 – Validation of immunological tolerance hypothesis
- 1957 – Clonal selection theory (Frank Macfarlane Burnet)
- 1957 – Discovery of interferon by Alick Isaacs and Jean Lindenmann[2]
- 1958–1962 – Discovery of human leukocyte antigens (Jean Dausset and others)
- 1959–1962 – Discovery of antibody structure (independently elucidated by Gerald Edelman and Rodney Porter)
- 1959 – Discovery of lymphocyte circulation (James Gowans)
- 1960 – Discovery of lymphocyte "blastogenic transformation" and proliferation in response to mitogenic lectins-phytohemagglutinin (PHA) (Peter Nowell)
- 1961-1962 Discovery of thymus involvement in cellular immunity (Jacques Miller)
- 1961- Demonstration that glucocorticoids inhibit PHA-induced lymphocyte proliferation (Peter Nowell)
- 1963 – Development of the plaque assay for the enumeration of antibody-forming cells in vitro by Niels Jerne and Albert Nordin
- 1963 Gell and Coombs classification of hypersensitivity
- 1964-1968 T and B cell cooperation in immune response
- 1965 – Discovery of lymphocyte mitogenic activity, "blastogenic factor" (Shinpei Kamakura) and (Louis Lowenstein) (J. Gordon) and (L.D. MacLean)
- 1965 – Discovery of "immune interferon" (gamma interferon) (E.F. Wheelock)
- 1965 – Secretory immunoglobulins

- 1967 – Identification of IgE as the reaginic antibody (Kimishige Ishizaka)
- 1968 – Passenger leukocytes identified as significant immunogens in allograft rejection William L. Elkins and Ronald D. Guttmann)
- 1969 – The lymphocyte cytolysis Cr51 release assay (Theodore Brunner) and (Jean- Charles Cerottini)
- 1971 – Peter Perlmann and Eva Engvall at Stockholm University invented ELISA
- 1972 – Structure of the antibody molecule
- 1973 – Dendritic Cells first described by Ralph M. Steinman
- 1974 - Immune Network Hypothesis (Niels Jerne)
- 1974 – T-cell restriction to MHC (Rolf Zinkernagel and (Peter C. Doherty)
- 1975 – Generation of monoclonal antibodies (Georges Köhler) and (César Milstein)[3]
- 1975 - Discovery of Natural Killer cells (Rolf Kiessling, Eva Klein, Hans Wigzell)
- 1976 – Identification of somatic recombination of immunoglobulin genes (Susumu Tonegawa)
- 1980-1983 – Discovery and characterization of interleukins, 1 and 2 IL-1 IL-2 (Robert Gallo, Kendall A.Smith, Tadatsugu Taniguchi)
- 1983 – Discovery of the T cell antigen receptor TCR (Ellis Reinherz) (Philippa Marrack) and (John
- Kappler)[4] (James Allison)
- 1983 – Discovery of HIV (Luc Montagnier)
- 1985-1987 – Identification of genes for the T cell receptor
- 1986 – Hepatitis B vaccine produced by genetic engineering
- 1986 – Th1 vs Th2 model of T helper cell function (Timothy Mosmann)
- 1988 – Discovery of biochemical initiators of T-cell activation: CD4 and CD8-p56lck complexes
- (Christopher E. Rudd)
- 1990 – Gene therapy for SCID
- 1991 – Role of peptide for MHC Class II structure (Scheherazade Sadegh-Nasseri & Ronald N. Germain)
- 1992- Discovery of transitional B cells (David Allman & Michael Cancro) [5][6]
- 1994 – 'Danger' model of immunological tolerance (Polly Matzinger)
- 1995 – James P. Allison describes the function of CTLA-4

- 1995 – Regulatory T cells (Shimon Sakaguchi)
- 1995 – First Dendritic cell vaccine trial reported by Mukherji et al.
- 1996 - 1998 – Identification of Toll-like receptors
- 2000 - Discovery of M1 and M2 macrophage subsets (Charles Mills)[7]
- 2001 – Discovery of FOXP3 – the gene directing regulatory T cell development
- 2005 – Development of human papillomavirus vaccine (Ian Frazer)
- 2010 – First immune checkpoint inhibitor, ipilimumab (anti-CTLA-4), is approved by the FDA for treatment of stage IV melanoma
- 2011 – Carl June reports first successful use of CAR T-cells for the treatment of CD19+ malignancies
- 2014 – A second class of immune checkpoint inhibitor (anti-PD-1) is approved by the FDA for the treatment
- of melanoma. Two different drugs, pembrolizumab and nivolumab are approved within months of each other.
- 2016 – Matthew M. Halpert first characterizes role of dendritic cell CTLA-4 in Th-1 immunity

HOST PARASITE RELATIONSHIPS

INTRODUCTION:

a) Healthy individuals are INFECTED and are being infected anew constantly.

b) Some of these organisms maybe PATHOGENS (more frequently among the transient flora group). Some among the normal flora may be OPPORTUNISTS.

c) Our relationship with microbes is very dynamic:

THERE IS A BALANCE BETWEEN:

The disease-causing properties of the microbes and the antimicrobial defenses of the host.

d) INFECTIOUS DISEASE is disease caused by pathogenic microorganisms. Pathogenic = disease causing}

Virulence = degree or intensity of pathogenicity

Disease = abnormal state, deviation from a state of wellness or health Contamination means that microorganisms are present.

Infection, when parasitic microorganisms increase in number either within or on the body of the host.

e) Whether or not you will catch a disease depends on:

1) YOU: your health, nutrition, immune status.
2) The pathogen's VIRULENCE
3) How TOXIC the organism is
4) How INVASIVE the organism is

II. THE NORMAL FLORA

* The human adult is estimated to have 10^{13} human cells and 10^{14} bacterial cells.

* In all honesty, we are a cylinder with a hole through the center. Just about every surface is colonized. There is a parse area:

THE STOMACH - was thought to be free of organisms -- but recently shown that some organisms can survive Helicobacter *pylori* This is probably not considered normal flora since it is now known to cause ulcers.

THE BLADDER and the LOWER REACHES OF THE RESPIRATORY TRACT. These areas can be transiently infected but normal clearance mechanisms exist to get rid of the intruders. We are colonized at birth with *Lactobacillus*.

Over time we acquire and establish populations of:

* COLIFORMS - (*Escherichia coli* and other enteric gram-negative rods) - intestines

* *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Proprionibacterium acnes* and other diptheroids - skin

* *Streptococci* (*viridans* and *pneumoniae*) as well as some anaerobes - mouth

* *Lactobacilli* - vagina

THE VIRULENCE FACTORS OF MICROORGANISMS

a) PATHOGENICITY vs. VIRULENCE

- Pathogenicity - The ability of an organism to cause a disease. A taxonomically significant attribute generally ascribed to a species.

Generally, organisms referred to as pathogens have a high probability of causing an infection (and more reasonably should be called frank pathogens!), but under unusual circumstances any microorganism, even a non-pathogen, might be capable of causing an infection.

Virulence - The degree of pathogenicity. An attribute generally ascribed to a strain.

IMMUNITY

immunity is the capability of multicellular organisms to resist harmful microorganisms. **Immunity** involves both specific and nonspecific components.

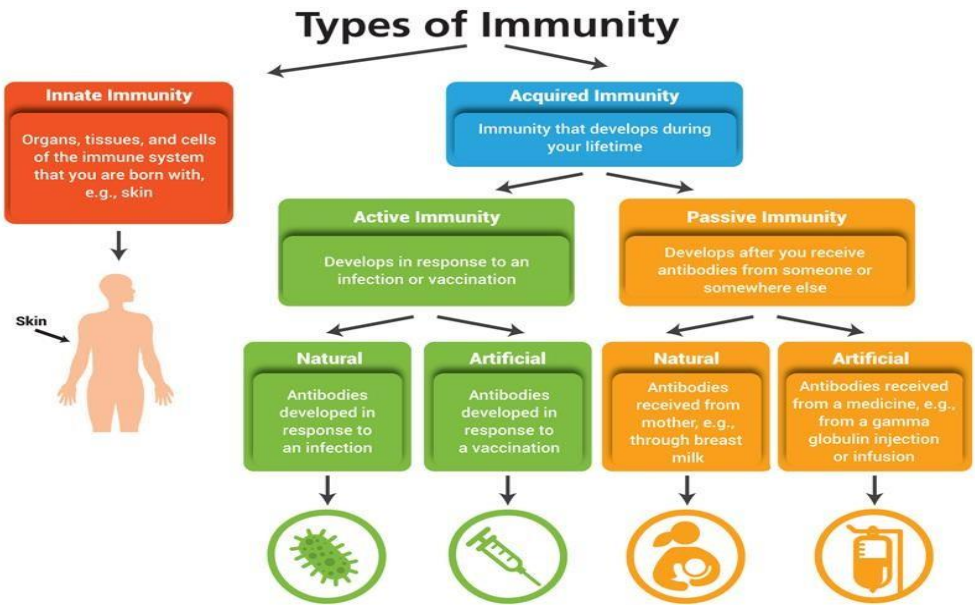

Fig. 1.1 Types of Immunity

INNATE IMMUNITY : Every day we are alive, humans encounter potentially harmful disease causing organisms, or “pathogens”, like bacteria or viruses. Yet most of us are still able to function properly and live life without constantly being sick. That’s because the human body requires a multilayered immune system to keep it running smoothly. The two main classes of the immune system are the innate immune system and the adaptive immune system, or “acquired immunity”. In this article, we’ll discuss the first line of defense: the innate immune system.

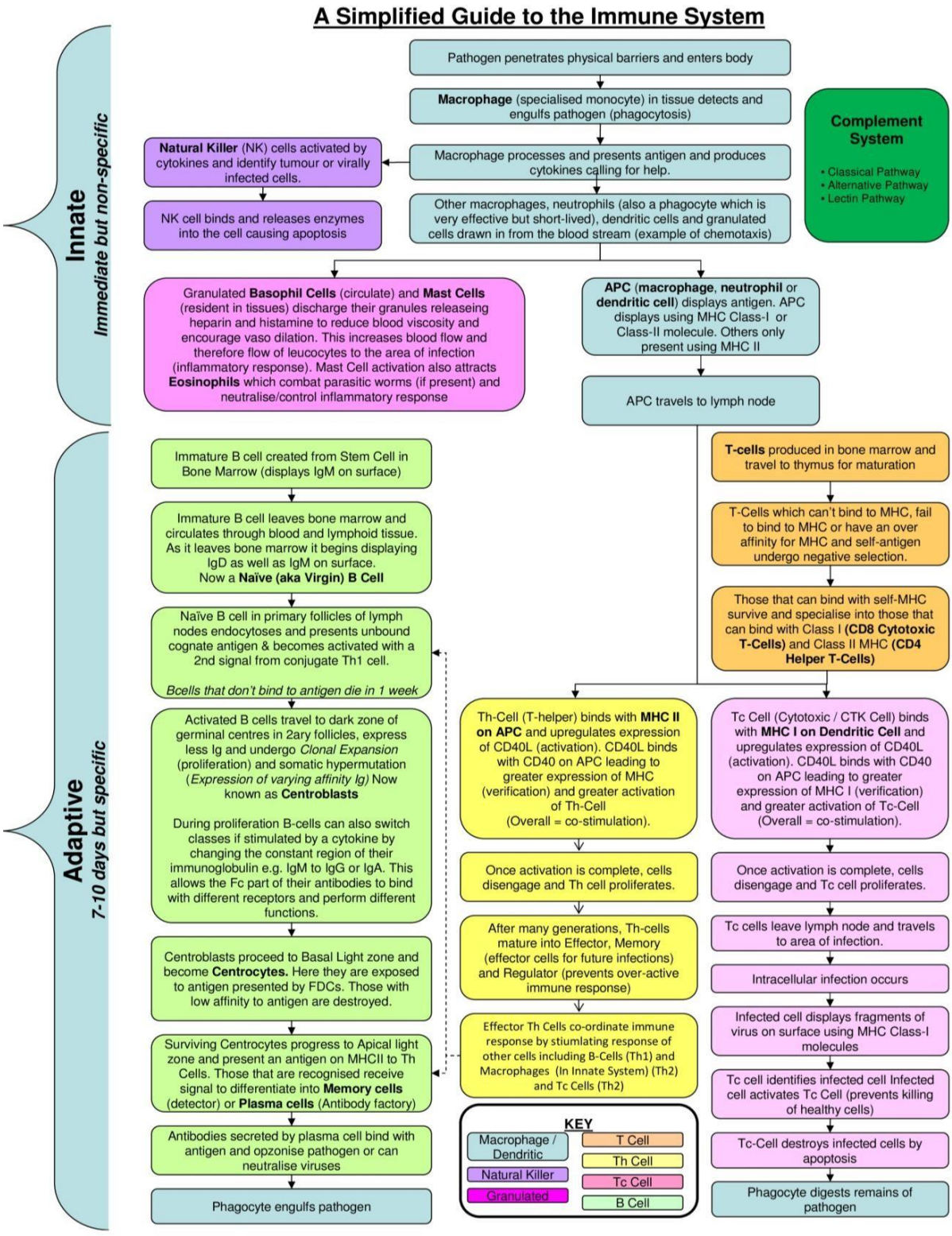

Fig.1.2 Innate and Adaptive Immunity

Self vs. Non-self: How does the body know?

In order to be effective, the immune system needs to be able to identify which particles are foreign, and which are a part of your body

Self refers to particles, such as proteins and other molecules, that are a part of, or made by, your body. They can be found circulating in your blood or attached to different tissues. Something that is self should not be targeted and destroyed by the immune system. The non- reactivity of the immune system to self-particles is called tolerance.

Non-self refers to particles that are not made by your body, and are recognized as potentially harmful. These are sometimes called *foreign bodies*. Non-self-particles or bodies can be bacteria, viruses, parasites, pollen, dust, and toxic chemicals. The non-self-particles and foreign bodies that are infectious or pathogenic, like bacteria, viruses, and parasites, make proteins called antigens that allow the human body to know that they intend to cause damage.

Antigens are anything that causes an immune response. Antigens can be entire pathogens, like bacteria, viruses, fungi, and parasites, or smaller proteins that pathogens express. Antigens are like a name tag for each pathogen that announce the pathogens' presence to your immune system. Some pathogens are general, whereas others are very specific. A general antigen would announce "I'm dangerous", whereas a specific antigen would announce "I'm a bacteria that will cause an infection in your gastrointestinal tract" or "I'm the influenza vir

Cytokines are molecules that are used for cell signaling, or cell-to-cell communication. Cytokines are similar to chemokines, wherein they can be used to communicate with neighboring or distant cells about initiating an immune response. Cytokines are also used to trigger cell trafficking, or movement, to a specific area of the body.

Chemokines are a type of cytokines that are released by infected cells. Infected host cells release chemokines in order to initiate an immune response, and to warn neighboring cells of the threat.

Innate Immune System

The innate immune system is made of defenses against infection that can be activated immediately once a pathogen attacks. The innate immune system is essentially made up of barriers that aim to keep viruses, bacteria, parasites, and other foreign particles out of your body or limit their ability to spread and move throughout the body. The innate immune system includes:

Anatomical Barriers such as skin, the gastrointestinal tract, the respiratory tract, the nasopharynx, cilia, eyelashes and other body hair.

Defense Mechanisms such as secretions, mucous, bile, gastric acid, saliva, tears, and sweat.

General Immune Responses such as inflammation, complement, and non-specific cellular responses. The inflammatory response actively brings immune cells to the site of an infection by increasing blood flow to the area. Complement is an immune response that marks pathogens for destruction and makes holes in the cell membrane of the pathogen.

The innate immune system is always general, or *nonspecific*, meaning anything that is identified as foreign or *non-self* is a target for the innate immune response. The innate immune system is activated by the presence of antigens and their chemical properties.

Cells of the Innate Immune System

There are many types of white blood cells, or *leukocytes*, that work to defend and protect the human body. In order to patrol the entire body, leukocytes travel by way of the circulatory system.

The following cells are leukocytes of the innate immune system:

- *Phagocytes*, or *Phagocytic cells*: Phagocyte means "eating cell", which describes what role phagocytes play in the immune response. Phagocytes circulate throughout the body, looking for potential threats, like bacteria and viruses, to engulf and destroy. You can think of phagocytes as security guards on patrol.

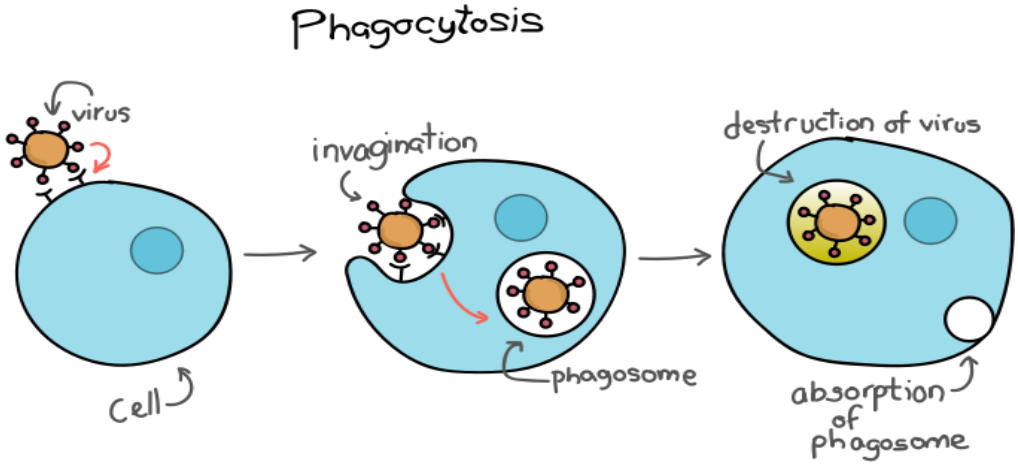

Fig. 1.3 Phagocytosis diagram explains how phagocytes know what to engulf, and how phagocytosis works.

- *Macrophages*: Macrophages, commonly abbreviated as "Mφ", are efficient phagocytic cells that can leave the circulatory system by moving across the walls of capillary vessels. The ability to roam outside of the circulatory system is important, because it allows macrophages to hunt pathogens with less limits. Macrophages can also release cytokines in order to signal and recruit other cells to an area with pathogens.
- *Mast cells*: Mast cells are found in mucous membranes and connective tissues, and are important for wound healing and defense against pathogens via the inflammatory response. When mast cells are activated, they release cytokines and granules that contain chemical molecules to create an *inflammatory cascade*. Mediators, such as histamine, cause blood vessels to dilate, increasing blood flow and

cell trafficking to the area of infection. The cytokines released during this process act as a messenger service, alerting other immune cells, like neutrophils and macrophages, to make their way to the area of infection, or to be on alert for circulating threats.

- *Neutrophils*: Neutrophils are phagocytic cells that are also classified as *granulocytes* because they contain granules in their cytoplasm. These granules are very toxic to bacteria and fungi, and cause them to stop proliferating or die on contact.

The bone marrow of an average healthy adult makes approximately 100 billion new neutrophils per day. Neutrophils are typically the first cells to arrive at the site of an infection because there are so many of them in circulation at any given time.

Eosinophils: Eosinophils are granulocytes target multicellular parasites. Eosinophils secrete a range of highly toxic proteins and free radicals that kill bacteria and parasites. The use of toxic proteins and free radicals also causes tissue damage during allergic reactions, so activation and toxin release by eosinophils is highly regulated to prevent any unnecessary tissue damage.

While eosinophils only make up 1-6% of the white blood cells, they are found in many locations, including the thymus, lower gastrointestinal tract, ovaries, uterus, spleen, and lymph nodes.

Basophils: Basophils are also granulocytes that attack multicellular parasites. Basophils release histamine, much like mast cells. The use of histamine makes basophils and mast cells key players in mounting an allergic response.

Natural Killer cells: Natural Killer cells (NK cells), do not attack pathogens directly. Instead, natural killer cells destroy infected host cells in order to stop the spread of an infection. Infected or compromised host cells can signal natural kill cells for destruction through the expression of specific receptors and antigen presentation.

Dendritic cells: Dendritic cells are antigen-presenting cells that are located in tissues, and can contact external environments through the skin, the inner mucosal lining of the nose, lungs, stomach, and intestines. Since dendritic cells are located in tissues that are common points for initial infection, they can identify threats and act as messengers for the rest of the immune system by antigen presentation. Dendritic cells also act as bridge between the innate immune system and the adaptive immune system.

The Complement System

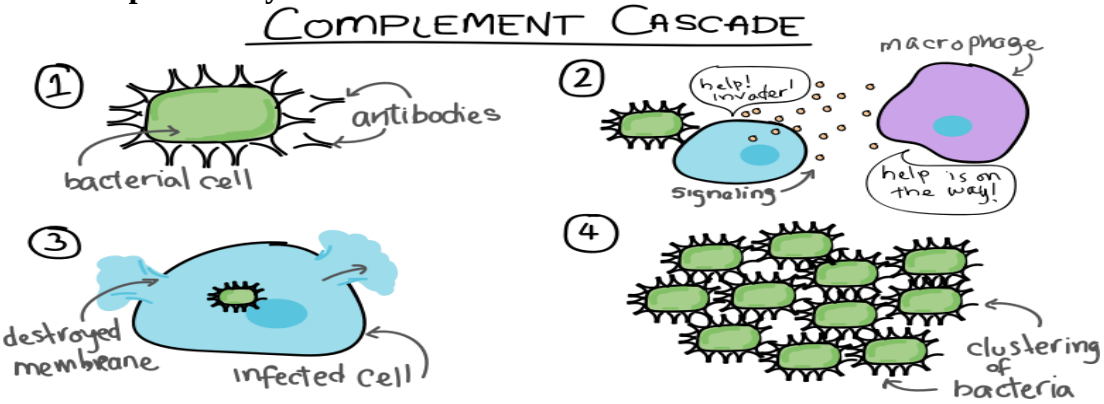

Fig. 1.4 Complement cascade activation

The complement system (also called the *complement cascade*) is a mechanism that *complements* other aspects of the immune response. Typically, the complement system acts as a part of the innate immune system, but it can work with the adaptive immune system if necessary.

The complement system is made of a variety of proteins that, when inactive, circulate in the blood. When activated, these proteins come together to initiate the complement cascade, which starts the following steps:

Opsonization: Opsonization is a process in which foreign particles are marked for phagocytosis. All of the pathways require an antigen to signal that there is a threat present.

Opsonization tags infected cells and identifies circulating pathogens expressing the same antigens.

Chemotaxis: Chemotaxis is the attraction and movement of macrophages to a chemical signal. Chemotaxis uses cytokines and chemokines to attract macrophages and neutrophils to the site of infection, ensuring that pathogens in the area will be destroyed. By bringing immune cells to an area with identified pathogens, it improves the likelihood that the threats will be destroyed and the infection will be treated.

Cell Lysis: Lysis is the breaking down or destruction of the membrane of a cell. The proteins of the complement system puncture the membranes of foreign cells, destroying the integrity of the pathogen. Destroying the membrane of foreign cells or pathogens weakens their ability to proliferate, and helps to stop the spread of infection.

Agglutination: Agglutination uses antibodies to cluster and bind pathogens together, much like a cowboy rounds up his cattle. By bringing as many pathogens together in the same area, the cells of the immune system can mount an attack and weaken the infection. Other innate immune system cells continue to circulate throughout the body in order to track down any

other pathogens that have not been clustered and bound for destruction.

The steps of the complement cascade facilitate the search for and removal of antigens by placing them in large clumps, making it easier for other aspects of the immune system to do their jobs. Remember that the complement system is a supplemental cascade of proteins that assists, or "complements" the other aspects of the innate immune system.

The innate immune system works to fight off pathogens before they can start an active infection. For some cases, the innate immune response is not enough, or the pathogen is able to exploit the innate immune response for a way into the host cells. In such situations, the innate immune system works with the adaptive immune system to reduce the severity of infection, and to fight off any additional invaders while the adaptive immune system is busy destroying the initial infection.

Adaptive Immunity

Have you ever wondered how your recovery time for the common cold, the flu, or small infections seems to get shorter after you've been exposed and successfully recovered the first time? The adaptive immune system, also called *acquired immunity*, uses specific antigens to strategically mount an immune response. Unlike the innate immune system, which attacks only based on the identification of general threats, the adaptive immunity is activated by exposure to pathogens, and uses an immunological memory to learn about the threat and enhance the immune response accordingly. The adaptive immune response is much slower to respond to threats and infections than the innate immune response, which is primed and ready to fight at all times.

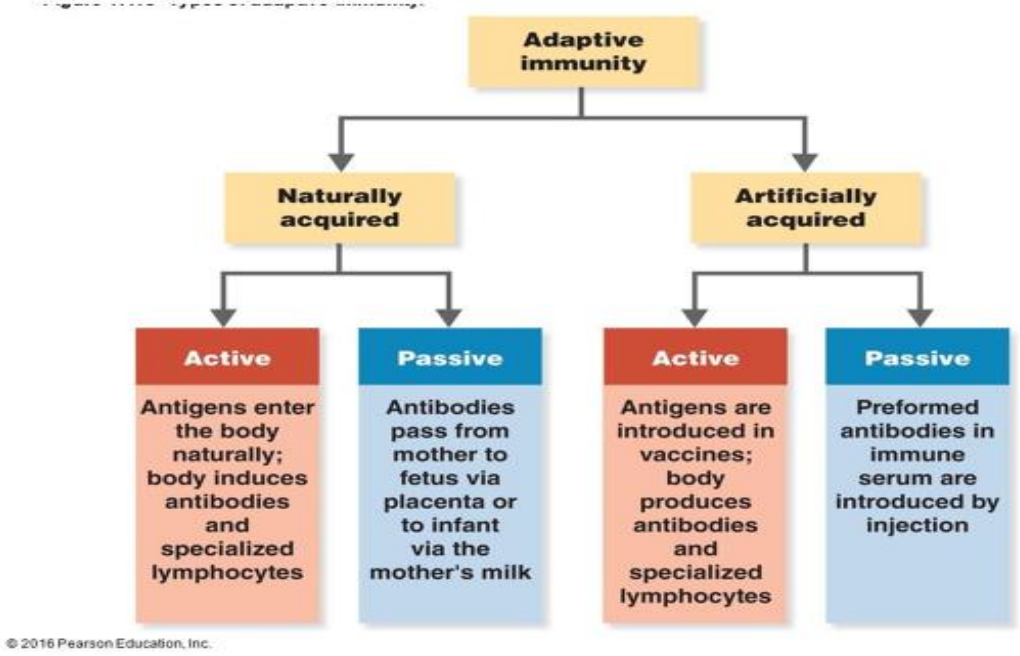

Fig. 1.5 Adaptive Immunity

Cells of the adaptive immune system

Unlike the innate immune system, the adaptive immune system relies on fewer types of cells to carry out its tasks: *B cells* and *T cells*.

Both B cells and T cells are lymphocytes that are derived from specific types of stem cells, called multipotent hematopoietic stem cells, in the bone marrow. After they are made in the bone marrow, they need to mature and become activated. Each type of cell follows different paths to their final, mature forms.

B cells

After formation and maturation in the bone marrow (hence the name "B cell"), the naive *B cells* move into the lymphatic system to circulate throughout the body. In the lymphatic system, naive B cells encounter an antigen, which starts the maturation process for the B cell. B cells each have one of millions of distinctive surface antigen-specific receptors that are inherent to the organism's DNA. For example, naive B cells express antibodies on their cell surface, which can also be called *membrane-bound antibodies*.

When a naive B cell encounters an antigen that fits or matches its membrane-bound antibody, it quickly divides in order to become either a *memory B cell* or an *effector B cell*, which is also called a *plasma cell*. Antibodies can bind to antigens directly.

The antigen must effectively bind with a naive B cell's membrane-bound antibody in order to set off *differentiation*, or the process of becoming one of the new forms of a B cell.

Memory B cells express the same membrane-bound antibody as the original naive B cell, or the "parent B cell". Plasma B cells produce the same antibody as the parent B cell, but they aren't membrane bound. Instead, plasma B cells can secrete antibodies. Secreted antibodies work to identify free pathogens that are circulating throughout the body. When the naive B cell divides and differentiates, both plasma cells and memory B cells are made.

B cells also express a specialized receptor, called the *B cell receptor (BCR)*. B cell receptors assist with antigen binding, as well as internalization and processing of the antigen. B cell receptors also play an important role in signaling pathways. After the antigen is internalized and processed, the B cell can initiate signaling pathways, such as cytokine release, 7 to communicate with other cells of the immune system. For more information on cell signalling, check out this article on cell-to-cell communication.

T cells

Once formed in the bone marrow, *T progenitor cells* migrate to the thymus (hence the name "T cell") to mature and become T cells. While in the thymus, the developing T cells start to express *T cell receptors (TCRs)* and other receptors called *CD4* and *CD8* receptors. All T cells express T cell receptors, and either CD4 or CD8, not both. So, some T cells will express CD4, and others will express CD8.

Unlike antibodies, which can bind to antigens directly, T cell receptors can only recognize antigens that are bound to certain receptor molecules, called *Major Histocompatibility Complex class 1 (MHCI)* and *class 2 (MHCII)*. These MHC molecules are membrane-bound surface receptors on *antigen-presenting cells*, like dendritic cells and macrophages. CD4 and CD8 play a role in T cell recognition and activation by binding to either MHCI or MHCII.

T cell receptors have to undergo a process called rearrangement, causing the nearly limitless recombination of a gene that expresses T cell receptors. The process of rearrangement allows for a lot of binding diversity. This diversity could potentially lead to accidental attacks against self cells and molecules because some rearrangement configurations can accidentally mimic a person's self molecules and proteins. Mature T cells should recognize only foreign antigens combined with self-MHC molecules in order to mount an appropriate immune response.

In order to make sure T cells will perform properly once they have matured and have been released from the thymus, they undergo two selection processes:

1. *Positive* selection ensures MHC restriction by testing the ability of MHCI and MHCII to distinguish between self and nonself proteins. In order to pass the positive selection process, cells must be capable of binding only self-MHC molecules. If these

cells bind nonself molecules instead of self-MHC molecules, they fail the positive selection process and are eliminated by apoptosis.

2. *Negative* selection tests for self tolerance. Negative selection tests the binding capabilities of CD4 and CD8 specifically. The ideal example of self tolerance is when a T cell will only bind to self-MHC molecules presenting a foreign antigen. If a T cell binds, via CD4 or CD8, a self-MHC molecule that isn't presenting an antigen, or a self-MHC molecule that presenting a self-antigen, it will fail negative selection and be eliminated by apoptosis.

These two selection processes are put into place to protect your own cells and tissues against your own immune response. Without these selection processes, autoimmune diseases would be much more common.

After positive and negative selection, we are left with three types of mature T cells: *Helper T cells*, *Cytotoxic T cells* and *T regulatory cells*.

- *Helper T* cells express CD4, and help with the activation of B cells, and other immune cells.
- *Cytotoxic T* cells express CD8, and are responsible for removing pathogens and infected host cells.
- *T* regulatory cells express CD4 and another receptor, called CD25. T regulatory cells help distinguish between self and nonself molecules, and by doing so, reduce the risk of autoimmune diseases.

Humoral vs. Cell Mediated Immunity

Immunity refers to the ability of your immune system to defend against infection and disease. There are two types of immunity that the adaptive immune system provides, and they are dependent on the functions of B and T cells, as described above.

Humoral immunity is immunity from serum antibodies produced by plasma cells. More specifically, someone who has never been exposed to a specific disease can gain humoral immunity through administration of antibodies from someone who has been exposed, and survived the same disease. "Humoral" refers to the bodily fluids where these free-floating serum antibodies bind to antigens and assist with elimination.

Cell-mediated immunity can be acquired through T cells from someone who is immune to the target disease or infection. "Cell-mediated" refers to the fact that the response is carried out by cytotoxic cells. Much like humoral immunity, someone who has not been exposed to a specific disease can gain cell-mediated immunity through the administration of cells from someone that

has been exposed, and survived the same disease. The T cells act to activate other immune cells, while the cells assist with the elimination of pathogens and infected host cells.

Immunological memory

Because the adaptive immune system can learn and remember specific pathogens, it can provide long-lasting defense and protection against recurrent infections. When the adaptive immune system is exposed to a new threat, the specifics of the antigen are memorized so we are prevented from getting the disease again. The concept of immune memory is due to the body's ability to make antibodies against different pathogens.

A good example of immunological memory is shown in vaccinations. A vaccination against a virus can be made using either active, but weakened or attenuated virus, or using specific parts of the virus that are not active. Both attenuated whole virus and virus particles cannot actually cause an active infection. Instead, they mimic the presence of an active virus in order to cause an immune response, even though there are no real threats present. By getting a vaccination, you are exposing your body to the antigen required to produce antibodies specific to that virus, and acquire a memory of the virus, without experiencing illness.

Some breakdowns in the immunological memory system can lead to autoimmune diseases. Molecular mimicry of a self-antigen by an infectious pathogen, such as bacteria and viruses, may trigger autoimmune disease due to a cross-reactive immune response against the infection. One example of an organism that uses molecular mimicry to hide from immunological defenses is *Streptococcus* infection.

Innate Immunity vs. Adaptive Immunity: A summary

The following chart compares and summarizes all of the important parts of each immune system:

Attribute	Innate Immunity	Adaptive Immunity
Response Time	Fast: minutes or hours	Slow: days

Specificity	Only specific for molecules and molecular patterns associated with general pathogens or foreign particles	Highly specific! Can discriminate between pathogen vs. non-pathogen structures, and miniscule differences in molecular structures
Major Cell Types	Macrophages, Neutrophils, Natural Killer Cells, Dendritic Cells, Basophils, Eosinophils	T cells, B cells, and other antigen presenting cells
Key Components	Antimicrobial peptides and proteins, such as toxic granules	Antibodies
Self vs. Nonself Discrimination	Innate immunity is based on self vs. nonself discrimination, so it has to be perfect	Not as good as the innate immune system, but still pretty good at determining which is which. Problems in self vs. nonself discrimination result in autoimmune diseases
Immunological Memory	None	Memory used can lead to faster response to recurrent or subsequent infections
Diversity and Customization	Limited: Receptors used are standard and only recognize antigen patterns. No new receptors are made to adapt the immune response	Highly diverse: can be customized by genetic recombination to recognize epitopes and antigenic determinants.

Difference Between Humoral and Cell Mediated Immunity

Main Difference – Humoral Immunity vs Cell mediated immunity

Humoral immunity and cell mediated immunity are two types of adaptive immunity. Adaptive immunity generates an antigen-specific immune response. During adaptive immunity, the antigen is first recognized through receptors of the lymphocytes, and immune cell clones are produced to attack that particular antigen. Humoral immunity is triggered by B cells while cell mediated immunity is triggered by T cells. The **main difference** between humoral and cell mediated immunity is that **antigen-specific antibodies are produced in humoral immunity whereas antibodies are not produced in cell mediated immunity**. Instead, T cells destroy the infected cells by inducing apoptosis.

Primary and Secondary Immunity

Primary Immunity – When first exposed to an antigen, the body usually takes several days

to respond and build up a large supply of antibodies. The number of antibodies will peak and then begin to decline.

Secondary Immunity – The production of Memory B or T Cells allows the cell to recognize the antigen much quicker if it is introduced again so the body will often be able to destroy the invading antigen before its numbers become great enough to initiate symptoms. Memory B cells rapidly divide and develop into plasma cells and the antibody levels in the body rise quickly and reach greater numbers. Active immunity lasts as long as clones of memory B and memory T cells are present

Sources of Specific Immunity – resistance to a disease causing organism or harmful substance

- **Inborn Immunity** – Immunity for certain diseases is inherited
- **Acquired Immunity** – immunity can be acquired through infection or artificially by medical intervention exposure to causative agent or antigen is not deliberate and occurs in the course of everyday living as exposure to a disease causing pathogen or allergen

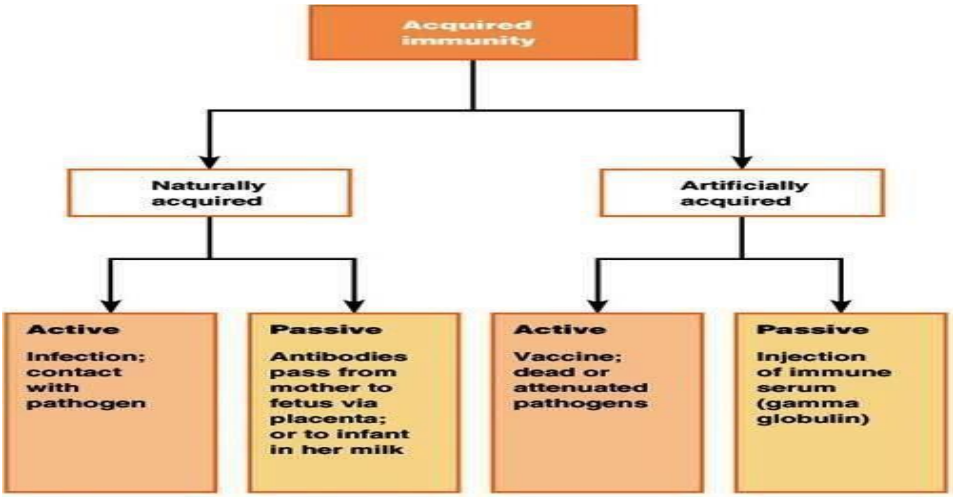

Fig. 1.6 Acquired Immunity

- **Active Exposure** – you develop your own antibodies – Immunity is long lived
- **Passive Exposure** – you receive antibodies from another source as infants receiving antibodies from mother's milk. This immunity is short-lived

Artificial Immunity or ***Immunization*** – exposure to causative agent or antigen is deliberate

- **Active Exposure** – injection of causative agent that has been weakened or killed such as a **vaccine** and you develop your own antibodies - Immunity is long lived

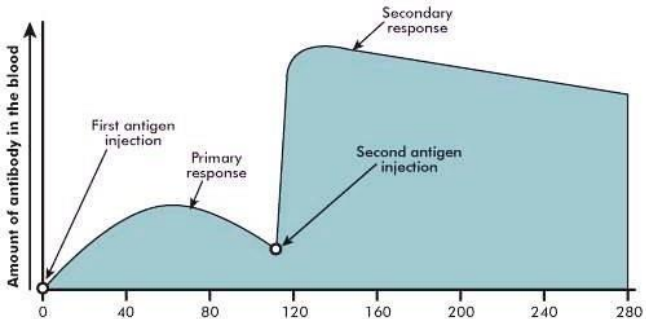

Fig. 1.7 Primary and secondary Immune response

What is Humoral Immunity

Humoral immunity is the immunity generated by circulating antibodies. It is a component of adaptive immunity, which generates specific immune responses to a particular foreign material. The extracellular spaces of the body are

protected by humoral immunity. Most pathogens that invade the body multiply in the extracellular spaces. Intracellular pathogens move from one cell to another through the extracellular space. Therefore, extracellular space is an important place to destroy pathogens. Antibodies are produced and secreted by plasma B cells. Typically, the activation of B cells occurs in T helper cells.

Antibodies destroy pathogens in three ways. They bind to the specific molecules on the surface of the pathogen, neutralizing the pathogen. This neutralization prevents the entering of the pathogen to the cells. It is also important to prevent bacterial toxins. The antibody-caught pathogens are subjected to **phagocytosis** by macrophages and other cells. This process is called **opsonization**. The binding of antibodies to the pathogens activates the complement system. The complement proteins bind to the antibody-bound pathogens and recruit phagocytic cells. The opsonization is shown in *figure 1*.

What is Cell Mediated Immunity

Cell mediated immunity is the immunity mediated by antigen-specific T cells. T cells are produced in the bone marrow and are matured in the thymus. After they enter the bloodstream, T cells occur can be found in the blood as well as in lymphoid tissue. The antigens should be presented on the surface of the antigen-presenting cells (APCs) along with the major histocompatibility complexes (MHC). Once T cells encounter an antigen, they proliferate and differentiate into armed effector cells. The cytotoxic T cells destroy the infected cells by inducing apoptosis. T helper cells stimulate plasma B cells to produce antibodies.

The IgG and IgM are the main two types of antibodies produced by T helper cells in response to plasma B cells. The memory T cells are differentiated T cells, but their action requires the activation by the specific antigen. The major characteristic feature of the cell mediated immunity is that it destroys intracellular pathogens. The cell mediated immunity is shown

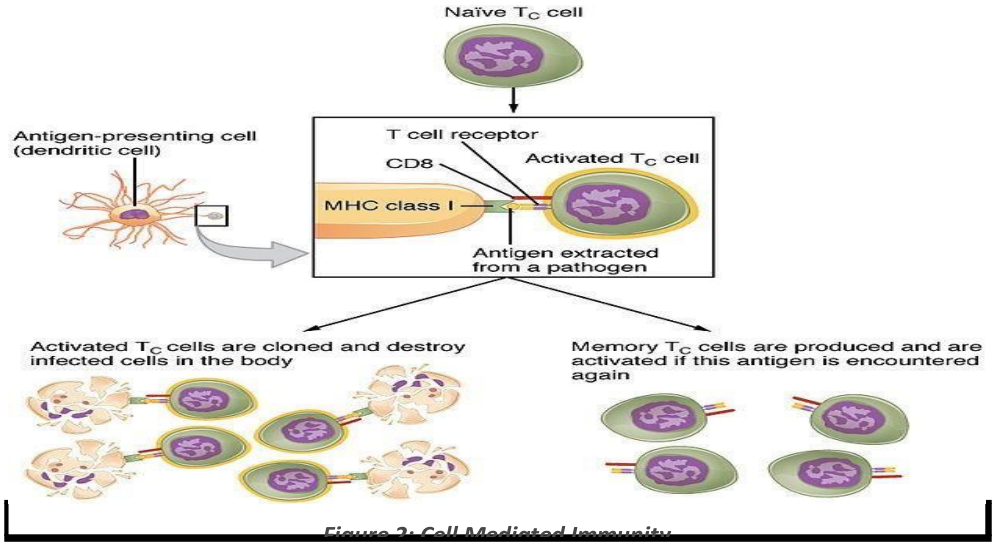

Figure 2: Cell Mediated Immunity

HUMORAL IMMUNITY
VERSUS
CELL MEDIATED IMMUNITY

HUMORAL IMMUNITY	CELL MEDIATED IMMUNITY
Humoral immunity refers to a component of the adaptive immunity where B cells secrete antibodies, which circulate in the blood as a soluble protein	Cell mediated immunity refers to the other component of the adaptive immunity, which is mediated by the activated, antigen-specific T cells
Mediated by B cells	Mediated by T cells
Mediated by T cells, B cells, and macrophages	Mediated by helper T cells, cytotoxic T cells, natural killer cells, and macrophages
Acts on extracellular microbes and their toxins	Acts on intracellular microbes such as viruses, bacteria, and parasites and tumor cells
Involves BCR receptors	Involves TCR receptors
Igα, Igβ, CD40, CD21, and Fc receptors are the accessory receptors	CD2, CD3, CD4, CD8, CD28, and integrins are the accessory receptors
Recognizes unprocessed antigens	Antigens are processed and presented by MHC complexes
Plasma B cells secrete antibodies	T cells secrete cytokines
Rapid	A delayed type hypersensitivity
Does not act on the tumor cells and transplants	Acts on tumor cells and transplants

Organs of the Lymphatic System

Lymph Nodes

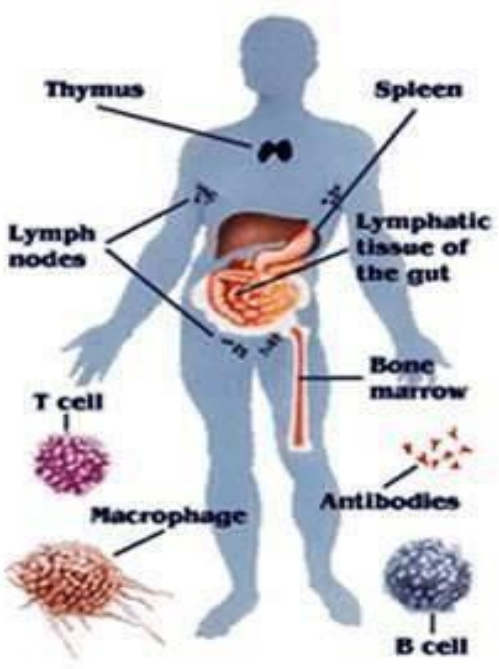

Fig. 1.8 Lymphoid organs

- Small (1- 25 mm) round structures found at points along lymphatic vessels that have fibrous connective tissue capsule with incoming and outgoing lymphatic vessels
- Each nodule contains sinus filled with lymphocytes and macrophages
- They occur in regions: auxiliary nodes in armpits and inguinal nodes in groin
- Occur singly or in groups of nodules:
  - **Tonsils** are located in back of mouth on either side
  - **Adenoids** on posterior wall above border of soft palate
  - **Peyer's patches** found within intestinal wall

Spleen

- Located in upper left ab dominal cavity just beneath diaphragm. Structure similar to lymph node; outer connective tissue divides organ into lobules with sinuses filled with blood
- Blood vessels of spleen can expand so spleen functions as blood reservoir making blood available in times of low pressure or oxygen need
- ***Red pulp*** containing RBCs, lymphocytes, and macrophages; functions to remove bacteria and worn-out red blood cells

- ***White pulp*** contains mostly lymphocytes
- Both help to purify the blood

Thymus

- Located along trachea behind sternum in upper thorax
- Larger in children; disappears in old age
- Divided into lobules where T lymphocytes mature
- Interior (medulla) of lobule secretes *thymosin* thought to aid T cells to mature

Red Bone Marrow

- Site of origin of all types of blood cells
- Five types of white blood cells (WBCs) function in immunity
- Stem cells continuously divide to produce cells that differentiate into various bloodcells
- Most bones of children have red blood marrow
- In adult, red marrow is found in the skull, sternum, ribs, clavicle, spinal column, femur, and humerus
- Red blood marrow has network of connective tissue where reticular cells produce reticular fibers; these plus stem cells fill sinuses; differentiated blood cells enter bloodstream at these sinuses

Immune tissue associated with various organs:

GALT—***gut-associated*** lymphatic tissue; comprised of lymphoid tissue (lymph nodules) in the intestinal wall containing lymphocytes, plasma cells and macrophages.

- The digestive tract is a very important part of the immune system and the intestine possesses the largest mass of lymphoid tissue in the body.

Lymphoid tissue in the gut comprises the following:

- ***Tonsils*** (Waldeyer's ring)
- ***Adenoids*** (Pharyngeal tonsils)
- ***Peyer's patches*** **–** lymphoid follicles in wall of small intestine
- Lymphoid aggregates in the appendix and large intestine
- Lymphoid tissue accumulating with age in the stomach
- Small lymphoid aggregates in the esophagus
- Diffusely distributed lymphoid cells and plasma cells in lining of the gut

MALT—***mucosa-associated*** lymphatic tissue; lymphoid tissue associated with the mucosa of the female reproductive tract, respiratory tract, etc.

SALT—***skin-associated*** lymphatic tissue; lymphatic tissue associated with the dermis of the skin.

Plan of Protection – ***Immunity*** is the ability to defend against infectious agents, foreign cells and abnormal cells eg. cancerous cells

- **1st Line of defense** – Block entry
- **2nd Line of Defense** – Fight Local Infections
- **3rd Line of Defense** – Combat Major Infections

Nonspecific and Specific Defense Systems - work together to coordinate their responses

Nonspecific (Innate) Response **- responds quickly, fights all invaders and consists of:**

- **First line of defense** – intact skin and mucosae and secretions of skin and mucous membranes prevent entry of microorganisms
- **Second line of defense** – phagocytic white blood cells, antimicrobial proteins, and other cells
- Inflammatory response process is key
- Inhibit invaders from spreading throughout the body

Specific Response (Adaptive) Response **- takes longer to react, works on specific types of invaders which it identifies and targets for destruction**

- **Third line of defense** – mounts attack against particular foreign substances
- Lymphocytes and Antibodies
- Works in conjunction with the nonspecific or innate system

Nonspecific (Innate) Response – fight all invaders

First line of defence – Non specific barriers to block entry

- Skin provides an impervious barrier – physical or mechanical barrier
- Mucous membranes line the entrances of the body and produce mucus which traps foreign particles and directs them out of the body – physical or mechanical barrier
- Nasal hairs trap dirt and dust while microscopic cilia line some mucous membranes helping to trap foreign particles
- Gastric juice, vaginal secretions and urine are acidic fluids which provide protection
- Natural flora (harmless bacteria) in the intestine and vagina prevent pathogens from growing
- Tears, saliva and sweat possess some anti-bacterial properties
- Cerumen or ear wax protects the ear canal by trapping dirt and dust particles

Second line of defense – Fight local infection with Inflammation Process

- Begins as soon as the first line of defense is violated
- The response is a non-specific, immediate, maximal response to the presence of any foreign organism or substance and involves no immunological memory
- **Phagocytosis** is an important feature of cellular innate immunity performed by cells called 'phagocytes' that engulf, or eat, pathogens or particles
- **Phagocytes** – types of immune cells involved in phagocytosis - Produced throughout life by the bone marrow
- **Scavengers** – remove dead cells and microorganisms
- **Complement proteins** activate other proteins in a domino fashion resulting in a cascade of reactions which attract phagocytes to the site of the invasion, bind to the surface of microbes to insure WBC's can phagocytize the microbe and produce holes in the bacterial cell walls and membranes
- The **Inflammation Process** releases ***histamines***causing redness, pain, swelling, and heat

Phagocytes and their Relatives

Neutrophils - kill bacteria

- 60% of WBCs
- 'Patrol tissues' as they squeeze out of the capillaries
- Large numbers are released during infections
- Short lived – die after digesting bacteria
- Dead neutrophils make up a large proportion of puss

Monocytes – are chief phagocytes found in the blood

- Made in bone marrow as ***monocytes*** and the circulate in the blood for 1-2 days before being called ***macrophages*** once they reach organs.

Macrophages - Found in the organs, not the blood

- Larger than neutrophils and long lived - involved in phagocytosis, release interferon and interleukin (which stimulates production of cells of the Specific Defense System)
- Macrophages also act as scavengers, ridding the body of worn-out cells and other debris by ingesting cellular debris, foreign material, bacteria and fungi
- Versatile cells that reside within tissues and produce a wide array of chemicals including enzymes, complement proteins, and regulatory factors such as interleukin 1
- ***Antigen-presenting cells*** that activate the adaptive immune system they display

antigens from the pathogens to the lymphocytes.

Basophils – are capable of ingesting foreign particles and produce heparin and histamine and which induce inflammation, are often associated with asthma and allergies

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response.

They are most often associated with allergy and anaphylaxis: for example, they release histamine – this is why anti-histamines help allergic reactions

Dendritic cells are phagocytes in tissues that are in contact with the external environment Located mainly in the skin, nose, lungs, stomach, and intestines (are in no way connected to the nervous system)

Dendritic cells serve as a link between the innate and adaptive immune systems, as they present antigens to T cells, one of the key cell types of the adaptive immune system

Eosinophils – weakly phagocytic of pathogens kill parasitic worms

NK cells (natural killer) - used to combat tumor cells or virus-infected cells

A class of lymphocytes which attack and induce cells to kill themselves (self-induced apoptosis)

Cellular components – B cells and T cells - lymphocytes which are white blood cells

Humoral (antibody-mediated response) defends against extracellular pathogens by binding to antigens and making them easier targets for phagocytes and complement proteins

Cell mediated immune response – defends against intracellular pathogens and cancer by binding to and lyzing the infected cells or cancer cells

Humoral or antibody-mediated response – termed anti-body mediated because B cells produce antibodies and Humoral because antibodies are released into the bloodstream

B cells - are produced and mature in the bone marrow – they possess a protein on the B cells outer surface known as the B cell receptor (BCR) which allows them to bind to a specific antigen.

Plasma B cells also known as plasma cells, plasmocytes, and effector B cells– they produce antibodies

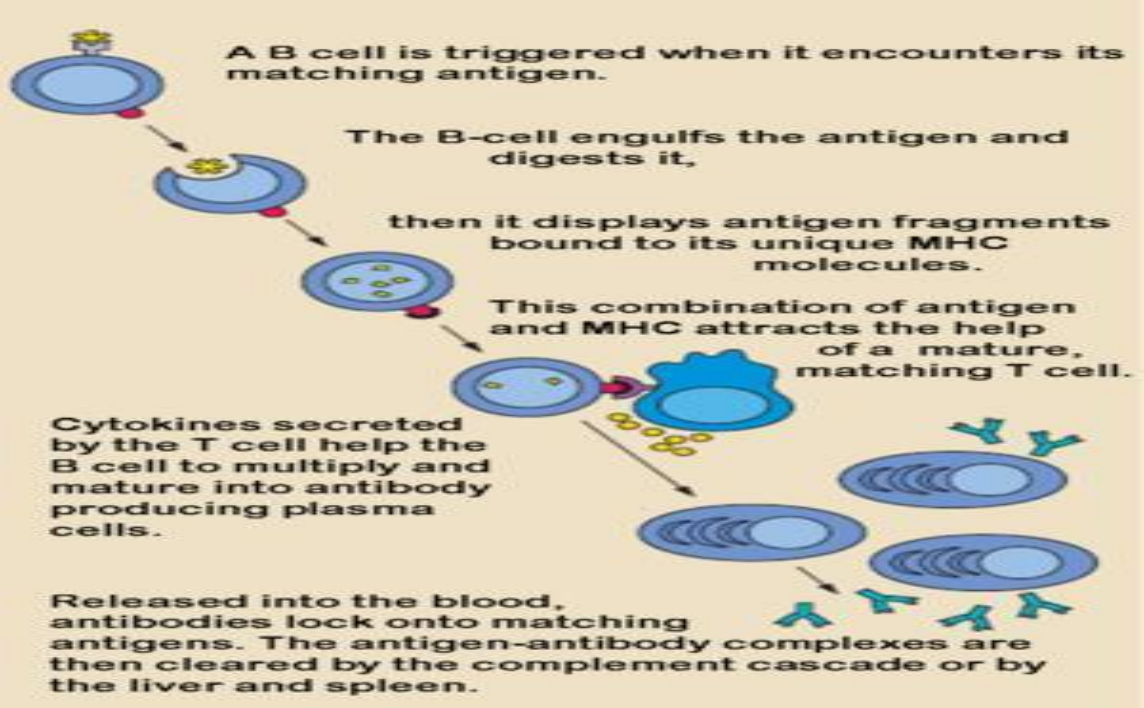

Fig. 1.9 B cell activation

Memory B cells – ready for the next invasion

B cell comes into contact with antigen on microbe, it attaches to the antigen and becomes an antigen-presenting, B cell with antigen-MHC complex, Helper T cell that binds to the complex Helper T secretes interleukin that stimulates mitosis in B cells so they multiply

Some B cells mature into **plasma cells** and other become

memory cells

The **plasma cells** produce **antibodies** also called **immunoglobins** – proteins which attach to the antigens

Antibodies can clump microbes for destruction, mark microbes for destruction by phagocytes, activate complement proteins that rupture/lyse microbe cell membranes or infected host cells

Memory B cells are stimulated to multiply but do not differentiate into plasma cells;

they provide the immune system with long-lasting memory.

***Cell-mediated immune response* (within the cell) -** does not involve antibodies but rather involves the activation of phagocytes, antigen-specific cytotoxic T-lymphocytes, and the release of various cytokines in response to an antigen

T cells – are produced in bone marrow but mature in the thymus gland T cells contribute to immune defenses in two major ways: some direct and regulate immune responses; others directly attack infected or cancerous cells.

Helper T cells – assist other white blood cells in the immunologic process including maturation of B cells into plasma cells and memory B cells and activation of T cells and macophages

Cytotoxic T cells – sometimes called killer T cells destroy virally infected cells and tumor cells and play a role in transplant rejection

Memory T cells –antigen-specific T cells the persist long-term after an infection has been resolved that will provide memory of past infection and earlier defense for new infection

Regulatory T cells – formally called **suppresser T cells** maintain balance by shutting down T-cell mediated immunity toward the end of an immune reaction – they are a self check built into the immune system to prevent excessive reactions. They play a key role in prevent autoimmunity.

Antigens are proteins or carbohydrate chain of a glycolprotein within in plasma membrane that the body recognizes as ***non-self***

The antigens on the cell membrane of the target or invader cell are recognized MHC (a protein marker on body's cell) binds to the antigen of the foreign cell forming an MHC complex. The MHC complex alerts the T cells about an invasion, macrophage, virgin B cell or cell infected by a microbe that displays the antigen on its membrane. The MHC complex activates the T cell receptor and the T cell secretes cytokines. The cytokines spur the production of more T cells. Some T cells mature into **Cytotoxic T cells** which attack and destroy cells infested with viruses or cancerous cells

Cytotoxic T cells or **Killer T cells (NKT)** share the properties of both T cells and natural killer (NK) cells. They are T cells with some of the cell-surface molecules of NK cells. The kills cancer cells, cells that are infected (particularly with viruses), or cells that are damaged in other ways -They have storage granules containing porforin and granzymes (proteins which perforates the cell membrane of the cell to be destroyed allowing water & salts to enter and rupture the cell). They and are implicated in disease progression of asthma and in protecting against some autoimmune diseases, graft rejection, and malignant tumors

Other T cells mature into **Helper T cells** which regulate immunity by increasing the response of other immune cells

Helper T cells secrete **cytokines** (messenger molecules) when exposed to antigens that causes more Helper T cells to be cloned, B cells to make antibodies and macrophages to destroy cells by phagocytosis

AID's virus attacks to Helper T cells so it inactivates the immune system control

Regulatory T cells will shut down T-cell mediated immunity when things are under

Memory T cells persist sometimes for life and protect in case of re-infection

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UNIT – II -Introduction to Immunology – SBMA1302

Antigen- Properties, Types and Determinants of Antigenicity

Antigen is a substance usually protein in nature and sometimes polysaccharide, that generates a specific immune response and induces the formation of a specific antibody or specially sensitized T cells or both.

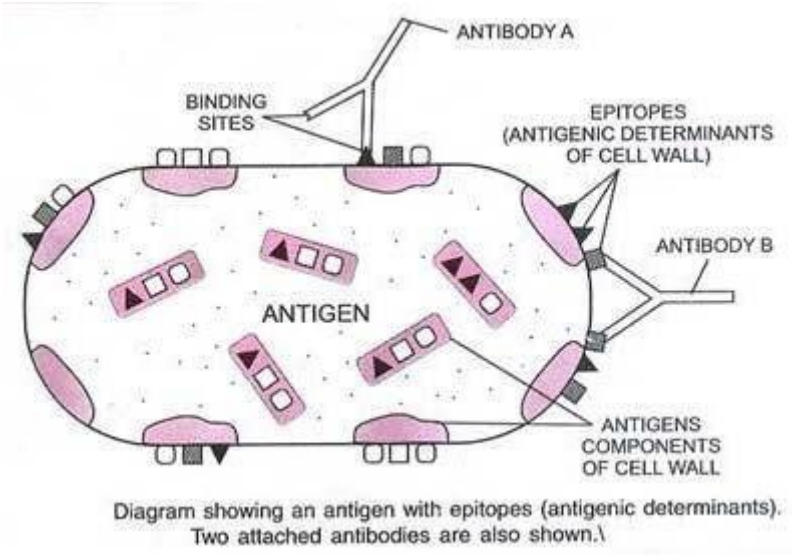


Fig. 2.1 Antigen with epitope

Although all antigens are recognized by specific lymphocytes or by antibodies, only some antigens are capable of activating lymphocytes. Molecules that stimulate immune responses are called I**mmunogens.**

Epitope is immunologically active regions of an immunogen (or antigen) that binds to antigen-specific membrane receptors on lymphocytes or to secreted antibodies. It is also called **antigenic determinants.**

Autoantigens, for example, are a person's own self antigens. Examples: Thyroglobulin, DNA, Corneal tissue, etc.

Alloantigens are antigens found in different members of the same species (the red blood cell antigens A and B are examples).

Heterophile antigens are identical antigens found in the cells of different species.

Examples: Forrssman antigen, Cross-reacting microbial antigens, etc.

Adjuvants are substances that are non-immunogenic alone but enhance the immunogenicity of any added immunogen.

Chemical Nature of Antigens (Immunogens)

A. Proteins

The vast majority of immunogens are proteins. These may be pure proteins or they may be glycoproteins or lipoproteins. In general, proteins are usually very good immunogens.

B. Polysaccharides

Pure polysaccharides and lipopolysaccharides are good immunogens.

C. Nucleic Acids

Nucleic acids are usually poorly immunogenic. However, they may become immunogenic when single stranded or when complexed with proteins.

D. Lipids

In general lipids are non-immunogenic, although they may be haptens.

Types of Antigen On the basis of order of their class (Origin)

1. Exogenous antigens

- These antigens enters the body or system and start circulating in the body fluids and trapped by the APCs (Antigen processing cells such as macrophages, dendritic cells, etc.)
- The uptakes of these exogenous antigens by APCs are mainly mediated by the phagocytosis
- Examples: bacteria, viruses, fungi etc
- Some antigens start out as exogenontigens, and later become endogenous (for example, intracellular viruses)

2. Endogenous antigens

- These are body's own cells or sub fragments or compounds or the antigenic products that are produced.
- The endogenous antigens are processed by the macrophages which are later accepted by the cytotoxic T – cells.
- Endogenous antigens include xenogenic (heterologous), autologous and idiotypic or allogenic (homologous) antigens.
- Examples: Blood group antigens, HLA (Histocompatibility Leukocyte antigens), etc. Report this ad

3. Autoantigens

- An autoantigen is usually a normal protein or complex of proteins (and sometimes DNA or RNA) that is recognized by the immune system of patients

suffering from a specific autoimmune disease

- These antigens should not be, under normal conditions, the target of the immune system, but, due mainly to genetic and environmental factors, the normal immunological tolerance for such an antigen has been lost in these patients.
- Examples: Nucleoproteins, Nucleic acids, etc.

On the basis of immune response

1. Complete Antigen or Immunogen

- Posses antigenic properties denovo, i.e. ther are able to generate an immune response by themselves.
- High molecular weight (more than 10,000)
- May be proteins or polysaccharides

2. Incomplete Antigen or Hapten

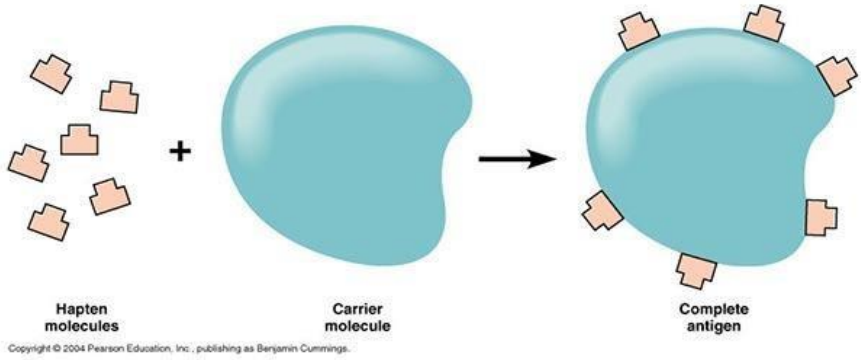


Fig. 2.2 Incomplete antigen

- These are the foreign substance, usually non-protein substances
- Unable to induce an immune response by itself, they require carrier molecule to act as a complete antigen.
- The carrier molecule is a non-antigenic component and helps in provoking the immune response. Example: Serum Protein such as Albumin or Globulin.
- Low Molecular Weight (Less than 10,000)
- Haptens can react specifically with its corresponding antibody.
- Examples: Capsular polysaccharide of pneumococcus, polysaccharide “C” of beta haemolytic streptococci, cardiolipin antigens, etc.

Determinants of Antigenicity

The whole antigen does not evoke immune response and only a small part of it induces B and T cell response. The small area of chemical grouping on the antigen molecule that determines specific immune response and reacts specifically with antibody is called an ***antigenic determinant.***

Property of antigens/ Factors Influencing Immunogenicity

Immunogenicity is determined by:

1. Foreignness

- An antigen must be a foreign substances to the animal to elicit an immune response.

2. Molecular Size

- The most active immunogens tend to have a molecular mass of 14,000 to 6,00,000 Da.
- Examples: tetanus toxoid, egg albumin, thyroglobulin are highly antigenic.
- Insulin (5700) are either non-antigenic or weakly antigenic.

3. Chemical Nature and Composition

- In general, the more complex the substance is chemically the more immunogenic it will be.
- Antigens are mainly proteins and some are polysaccharides.
- It is presumed that presence of an aromatic radical is essential for rigidity and antigenicity of a substance.

4. Physical Form

- In general particulate antigens are more immunogenic than soluble ones.
- Denatured antigens are more immunogenic than the native form.

5. Antigen Specificity

- Antigen Specificity depends on the specific actives sites on the antigenic molecules (Antigenic determinants).
- Antigenic determinants or epitopes are the regions of antigen which specifically binds with the antibody molecule.

6. Species Specificity

- Tissues of all individuals in a particular species possess, species specific antigen.
- Human Blood proteins can be differentiated from animal protein by specific antigen- antibody reaction.

7. Organ Specificity

- Organ specific antigens are confined to particular organ or tissue.
- Certain proteins of brain, kidney, thyroglobulin and lens protein of one species share specificity with that of another species.

8. Auto-specificity

- The autologous or self antigens are ordinarily not immunogenic, but under certain circumstances lens protein, thyroglobulin and others may act as ***autoantigens.***

9. Genetic Factors

- Some substances are immunogenic in one species but not in another. Similarly, some substances are immunogenic in one individual but not in others (i.e. responders and non- responders).
- The species or individuals may lack or have altered genes that code for the receptors for antigen on B cells and T cells.
- They may not have the appropriate genes needed for the APC to present antigen to the helper T cells.

10. Age

- Age can also influence immunogenicity.
- Usually the very young and the very old have a diminished ability to elicit and immune response in response to an immunogen.

11. Degradability

- Antigens that are easily phagocytosed are generally more immunogenic.
- This is because for most antigens (T-dependant antigens) the development of an immune response requires that the antigen be phagocytosed, processed and presented to helper T cells by an antigen presenting cell (APC).

12. Dose of the antigen

- The dose of administration of an immunogen can influence its immunogenicity.
- There is a dose of antigen above or below which the immune response will not be optimal.

13. Route of Administration

- Generally the subcutaneous route is better than the intravenous or intragastric routes.
- The route of antigen administration can also alter the nature of the response.
- Antigen administered intravenously is carried first to the spleen, whereas antigen administered subcutaneously moves first to local lymph nodes.

14. Adjuvants

- Substances that can enhance the immune response to an immunogen are called adjuvants.
- The use of adjuvants, however, is often hampered by undesirable side effects

such as fever and inflammation.

- Example: aluminum hydroxide.

Superantigens

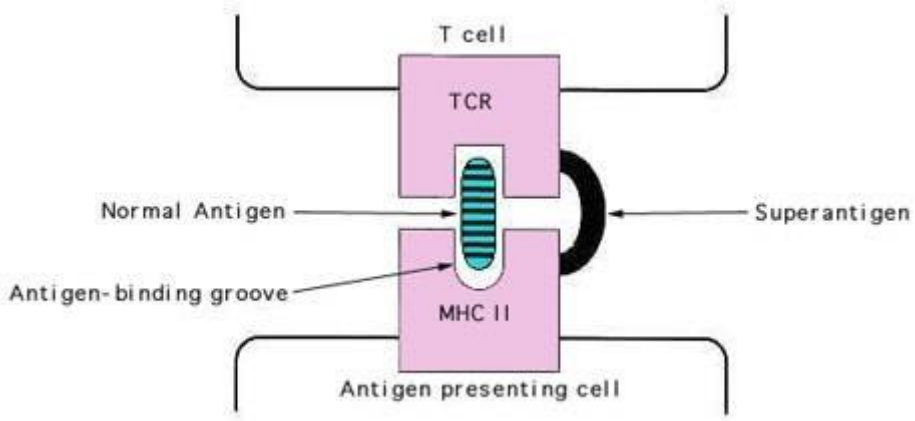


Fig. 2.3 Binding of super antigen

- When the immune system encounters a conventional T-dependent antigen, only a small fraction (1 in 104 -105) of the T cell population is able to recognize the antigen and become activated (monoclonal/oligoclonal response).
- However, there are some antigens which polyclonally activate a large fraction of the T cells (up to 25%). These antigens are called superantigens.
- Examples of superantigens include: Staphylococcal enterotoxins (food poisoning), Staphylococcal toxic shock toxin (toxic shock syndrome), Staphylococcal exfoliating toxins (scalded skin syndrome) and Streptococcal pyrogenic exotoxins (shock).
- Although the bacterial superantigens are the best studied there are superantigens associated with viruses and other microorganisms as well.
- The diseases associated with exposure to superantigens are, in part, due to hyper activation of the immune system and subsequent release of biologically active cytokines by activated T cells.

Antibody- Structure, Classes and Functions

Antibody (Ab) also know as Immunoglobulin (Ig) is the large Y shaped protein produced by the body's immune system when it detects harmful substances, called antigens like bacteria and viruses. The production of antibodies is a major function of the immune system and is carried

out by a type of white blood cell called a B cell (B lymphocyte), differentiated B cells called plasma cells. The produced antibodies bind to specific antigens express in external factors and cancer cells.

Structure of Antibody

Antibodies are heavy (~150 kDa) globular plasma proteins. The basic structure of all antibodies are same. There are four polypeptide chains: two identical **heavy chains** and two identical **light chains** connected by disulfide bonds. Light Chain (L) consists polypeptides of about 22,000 Da and Heavy Chain (H) consists larger polypeptides of around 50,000 Da or more. There are five types of Ig **heavy chain** (in mammal) denoted by the Greek letters: α, δ, ε, γ, and μ. There are two types of Ig **light chain** (in mammal), which are called lambda (λ) and kappa (κ). An antibody is made up of a variable region and a constant region, and the region that changes to various structures depending on differences in antigens is called the **variable region**, and the region that has a constant structure is called the **constant region.**

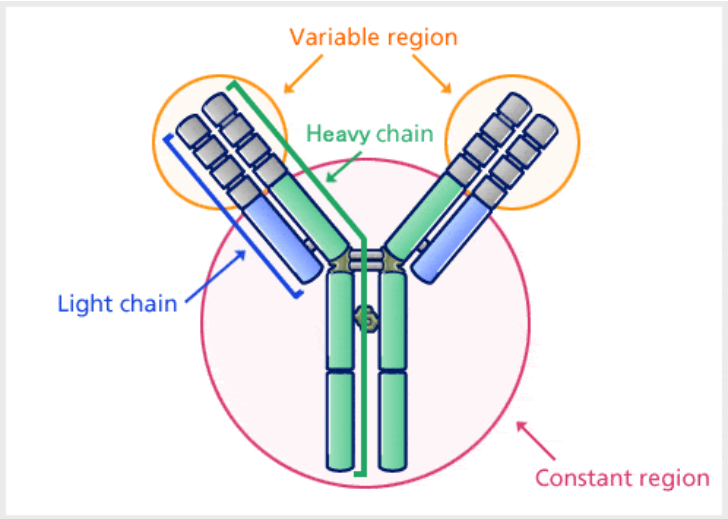


Fig. 2.4 Structure of Immunoglobulin

Each heavy and light chain in an immunoglobulin molecule contains an amino-terminal variable (V) region that consists of 100 to 110 amino acids and differ from one antibody to another. The remainder of each chain in the molecule – the constant (C) region exhibits limited variation that defines the two light chain subtypes and the five heavy chains subclasses. Some heavy chains (α, δ, γ) also contain a proline-rich hinge region. The amino terminal portions, corresponding to the V regions, bind to antigen; effector functions are mediated by the carboxy-terminal domains. The ε and μ heavy chains, which lack a hinge region, contain an additional domain in the middle of the molecule. CHO denotes a carbohydrate group linked to the heavy

chain.

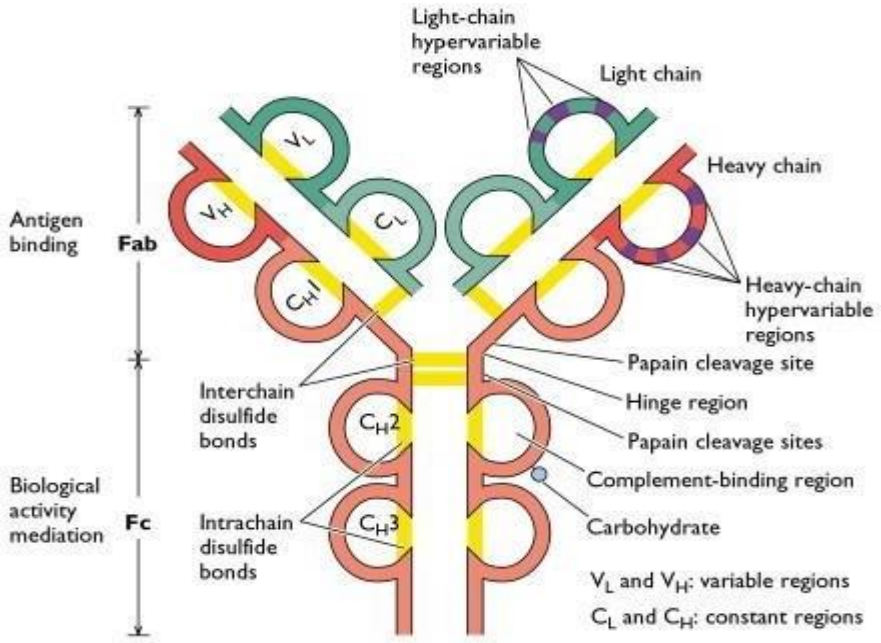


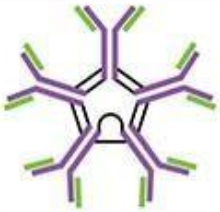
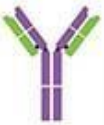
Fig. 2.5 Structure of Immunoglobulin domain

Classes/Types of Antibody

Serum containing antigen-specific antibodies is called antiserum. The 5 types – IgG, IgM, IgA, IgD, IgE – (isotypes) are classified according to the type of heavy chain constant region, and are distributed and function differently in the body.

There are five immunoglobulin classes of antibody molecules found in serum: IgG, IgM, IgA, IgE and IgD. They are distinguished by the type of heavy chain they contain.

Table 2.1 Deferent classes of Immunoglobulin

The Five Immunoglobulin (Ig) Classes					
	IgM pentamer	IgG monomer	Secretory IgA dimer	IgE monomer	IgD monomer
					
Heavy chains	μ	γ	α	ε	δ
Number of antigen binding sites	10	2	4	2	2
Molecular weight (Daltons)	900,000	150,000	385,000	200,000	180,000
Percentage of total antibody in serum	6%	80%	13%	0.002%	1%
Crosses placenta	no	yes	no	no	no
Fixes complement	yes	yes	no	no	no
Fc binds to		phagocytes		mast cells and basophils	
Function	Main antibody of primary responses, best at fixing complement; the monomer form of IgM serves as the B cell receptor	Main blood antibody of secondary responses, neutralizes toxins, opsonization	Secreted into mucus, tears, saliva, colostrum	Antibody of allergy and antiparasitic activity	B cell receptor

Functions of Antibody

1. IgG provides long term protection because it persists for months and years after the prescence of the antigen that has triggered their production.
2. IgG protect against bacteris, viruses, neutralise bacterial toxins, trigger compliment protein systems and bind antigens to enhance the effectiveness of phagocytosis.
3. Main function of IgA is to bind antigens on microbes before they invade tissues. It aggregates the antigens and keeps them in the secretions so when the secretion is expelled, so is the antigen.
4. IgA are also first defense for mucosal surfaces such as the intestines, nose, and lungs.
5. IgM is involved in the ABO blood group antigens on the surface of RBCs.
6. IgM enhance ingestions of cells by phagocytosis.

7. IgE bind to mast cells and basophils wich participate in the immune response.
8. Some scientists think that IgE's purpose is to stop parasites.
9. IgD is present on the surface of B cells and plays a role in the induction of antibody production.

IgG antibody structure and function

Immunoglobulin G (IgG) antibodies are large globular proteins with a molecular weight of about 150 kDa made of four peptide chains. It contains two identical γ (gamma) heavy chains of about 50 kDa and two identical light chains of about 25 kDa, thus a tetrameric quaternary structure.

IgG provides long term protection because it persists for months and years after the prescence of the antigen that has triggered their production. IgG protect against bacteria, viruses, neutralise bacterial toxins, trigger compliment protein systems and bind antigens to enhance the effectiveness of phagocytosis.

IgM antibody structure and function

Immunoglobulin M (IgM) antibodies are constructed of five or six units (i.e. mostly as pentamers but also hexamers occur) which are each comprised of two heavy-chains (μ- chains) and two light chains, bound together by disulfide bonds and a so-called J-chain.

IgM is involved in the ABO blood group antigens on the surface of RBCs. IgM enhance ingestions of cells by phagocytosis.

IgA antibody structure and function

Immunoglobulin A (IgA) antibodies consist of heavy (H) and light (L) chains. Each H chain is comprised of the constant region (Cα1, Cα2, Cα3), hinge region and the Variable (V) region. Light chains consists of the CL and Vκ or Vλ elements.

Main function of IgA is to bind antigens on microbes before they invade tissues. It aggregates the antigens and keeps them in the secretions so when the secretion is expelled, so is the antigen. IgA are also first defense for mucosal surfaces such as the intestines, nose, and lungs.

IgE antibody structure and function

Immunoglobulin E (IgE) antibodies have only been found in mammals. IgE is synthesized by plasma cells. Monomers of IgE consist of two heavy chains (ε chain) and two light chains, with the ε chain containing 4 Ig-like constant domains (Cε1-Cε4). IgE bind to mast cells and basophils which participate in the immune response. Some scientists think that IgE's purpose is to stop parasites.

IgD antibody structure and function

Immunoglobulin D (IgD) antibodies are expressed in the plasma membranes of immature B-

lymphocytes. IgD is also produced in a secreted form that is found in small amounts in blood serum. IgD plays a role in the induction of antibody production.

Antibody formats:

Chimeric antibodies can be generated by fairly straightforward genetic engineering, by joining the immunoglobulin (Ig) variable regions of a selected mouse hybridoma to human Ig constant regions, and be used as such or as a first stage towards further humanization.

Anti-idotypic antibody is an antibody that binds to the idiotype of another antibody. An Idiotype (ID) actually consists of multiple antigenic determinants, each of which is an idiotope. The antigenic determinants or idiotopes can reside in the heavy chain component of the V region, in its light chain component, or they may consist of a surface made up of parts of both chains.

The bispecific antibody can override the specificity of an effector cell for its natural target and redirect it to kill a target that it would otherwise ignore. Different cytotoxic cells express different triggering molecules (receptors). Thus, by varying the specificities of target and effector binding domains a variety of effector responses can be directed against most types of target cells. Alternatively, the full range of effector functions (i.e. ADCC, phagocytosis, complement activation and extended serum half-life) can be conferred by targeting one binding specificity to serum immunoglobulin.

Recombinant antibodies are monoclonal antibodies produced by recombinant DNA technology. Owing to their high specificity, sensitivity and reproducibility, recombinant antibodies are widely used in biomedical science and medicine.

Antigenic Determinants on Immunoglobulins:

Since antibodies are glycoproteins,they can themselves function as potent immunogens to induce an antibody response.Such anti-Ig antibodies are powerful tools for the study of B- cell development and humoral immune responses.The antigenic determinants, or epitopes, on immunoglobulin molecules fall into three major categories:isotypic,allotypic,and idiotypic determinants,which are located in characteristic portions of the molecule.

Isotypic Determinants:

Isotype determinants are consonant-region determinants that collectively define each heavy-chain class and sub class and each light-chain type and subtype within a species.Each isotype is encoded by a separate consonant region gene,and all members of a species carry the same consonant-region genes(which may include multiple alleles).Within a species,each normal individual will express all isotypes.Therefore,when an antibody from one species,each normal individual will express all isotypes in the serum.Different species inherit different constant-region genes and therefore express different isotypes.Therefore,when an antibody from one

species is injected into another species,the isotypic determinants will be recognized as foriegn,inducing an antibody response to the isotypic determinants on the foriegn antibody.Anti-isotype antibody is routinely used for research purposes to determine the class or subclass of serum antibody produced during an immune response or to characterize the class of membrane-bound antibody present on B cells.

Allotypic Determinants:

Although all members of a species inherit the same set of isotype genes,multiple alleles exist for some of the genes.These alleles encode subtle amino acid differences,called allotypic determinants displayed by an antibody determines its allotypes.In humans,allotypes have been characterized for all IgG subclasses,for one IgA subclass,and for the light chain.The gamma chain allotypes are referred to as Gm markers.At least 25 different Gm allotypes have been identified;they are designated by the class and subclass followed by the allele number,for example,G1m(1),G2m(23),G3m(11),G4m(4a). Of the two IgA subclasses,only the IgA2 subclass has allotypes,as A2m(1) andA2m(2). The light chain has three allotypes.Each of these allotypic determinants represents differences in one to four amino acids that are encoded by different alleles.

Idiotypic Determinants:

The unique amino acid sequence of the Heavy and light domains of a given antibody can function not only as an antigen-binding site but also as a set of antigenic determinants.The idiotypic determinants arise from the sequence of the heavy and light chain variable regions. Each individual antigenic determinant of the variable region is referred to as an idiotope.In some cases an idiotope maybe the actual antigen-binding site,and in some cases an idiotope may comprise variable region sequences outside of the antigen-binding site.Each antibody will present multiple idiotopes;the sum of the individual idiotopes is called idiotype of the antibody. Because the antibodies produced by individual B cells derived from the clone have identical variable-region sequences,they all have the same idiotype.Anti-idiotype antibody is produced by injecting antibodies that have minimal variation in their isotypes and allotypes ,so that the idiotypic differences can be recognized.Often a homogenous antibody such as myeloma protein or monoclonal antibody is used.Injection of such an antibody into a recipient who is genetically identical to the donor will result in formation of anti-idiotype antibody to the idiotypic determinants.

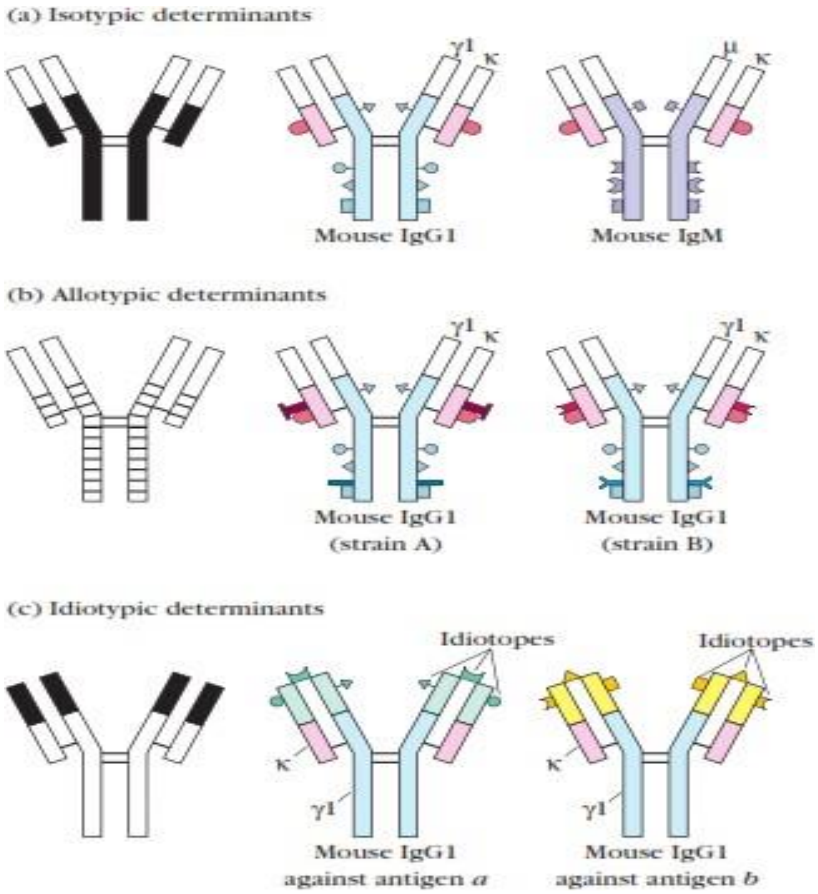


Fig. 2.6 Antigenic determinant

Complement Pathways: Types, Functions and Regulation

The complement system is a part of the immune system, consists of a series of proteins that interact with one another in a highly regulated manner, in order to eliminate pathogens. It helps antibodies and phagocytic cells to clear pathogens and damaged cells; promote inflammation and attack pathogen's plasma membrane. Proteins that take part in the complement system are called complements that collectively work as a **biological cascade**; the sequence of reactions, each being the catalyst for the next.

Jules Bordet (1895) identified complements as heat-sensitive components in the blood, bearing non-specific antimicrobial activity.

Complements are soluble proteins and glycoproteins mostly **produced by hepatocytes**. More than 20 types of complements are present in serum, found circulating normally in human body in inactive forms *(called as zymogens or proenzymes)*. Complement activation is triggered by an antibody when it is bound to the antigen. It can also be triggered by some components of

innate immunity. Thus, the **complement system works in both innate and acquired immunity.**

Complements are activated only during inflammatory reactions. During the inflammation, more amount of complements reaches to the interstitial area of the infected tissue through dilated blood vessels, which are then activated by proteolytic cleavage; this exposes the active site of the complements.

Complements are mainly **denoted by the capital letter *C*** with numbers; like, C1, C2, C3, and so on. Some have only alphabet, like, *B*, *D*. Some are simply represented by names, like, homologous restriction factor.

C1 has three sub-units; C1q, C1r and C1s. C2-C5 have two components, *a* and *b*. **Larger subunits are denoted by *b*** whereas the **smaller are denoted by *a*** *(except C2a, which is larger than C2b)*.

Complement Activation and cell lysis

The complement activation occurs via three pathways; which are:

1. **Classical pathway**
2. **Alternative pathway**
3. **Lectin pathway (or mannose binding lectin pathway)**

The early step of complement system varies in different pathways. However, all the pathways form enzyme complexes; C3 convertase, which cleaves C3 into C3a and C3b; and the C5 convertase, which cleaves C5 into C5a and C5b.C3b, thus formed, binds C3 convertase to form C5 convertase.

C5 convertase, generated by the alternative, classical, or lectin pathway, initiates the activation of late components of the complement system to form membrane attack complex (MAC) and ultimately kills the pathogen.

This occurs through three pathways; **Classical pathway**, activated by antigen-antibody reaction, **Alternative pathway**, activated on microbial cell surfaces, and **Mannose binding Lectin pathway**, activated by a plasma lectin that binds to mannose residues on microbes.

1. Classical Pathway

The classical pathway begins with the formation of antigen-antibody complex (immune complex). When an antigen enters the body, the antibody (IgM/IgG) binds to it. This induces conformational changes in the Fc portion of the antibody which exposes a binding site for C1

protein. Hence, the antibody activates the complement system only when bound to an antigen.

C1 is a large, multimeric, protein complex composed of one molecule of C1q and two molecules each of C1r and C1s subunits. C1q binds to the antigen bound antibody (Fc portion). C1r and C1s are proteases which help to cleave C4 and C2.

The immune complex bound to C1 calls another protein C4 which is cleaved into C4a and C4b. C4a goes away whereas activated C4b attaches to the target surface near C1q. Now, C4b attracts C2 which is also cleaved into C2a and C2b. C2a binds C4b forming the C4b2a complex whereas C2b goes away. The active C4bC2a activates C3. The C4b2a complex is also known as **C3 convertase** as this converts C3 into an active form by separating C3a and C3b. One molecule of C4b2a can cleave a large number of C3 molecules. C3b binds to the microbial surface or to the convertase itself.

C3b when binds to C3 convertase forms C4bC2aC3b (**C5 convertase**) which activates C5.

C5 convertase cleaves C5 into C5a and C5b. C5a diffuses away but C5b is stabilized by binding C6. Then C5bC6 binds to C7. C5bC6C7 complex is then inserted into the phospholipid bilayer of the cell membrane which further binds C8. These all (C5b678) activate C9 to form a macromolecular structure called the **membrane attack complex (MAC)**. This makes hole in the bacterium, as a result, the intracellular contents leak out and unwanted substances get in. Thus, the cell cannot maintain its osmotic stability and the lysis occurs by an influx of water and loss of electrolytes.

This is more effective in Gram negative bacteria than in Gram positive bacteria because MAC formation is easy in the outer membrane in Gram negatives whereas it is difficult in the rigid thick layer of peptidoglycan in Gram positives.

Some of the C3b molecules do not associate with C4b2a; instead these molecules coat immune complexes or microbial cell surfaces and work as opsonins. This process is called opsonization in which opsonin molecule binds one side to the particulate matter i.e. in bacteria, tumor cell, RBC and on the other side they bind to the receptor of phagocytic cell(like, neutrophils and macrophages) which enhance the process of phagocytosis.

Smaller complement subunits diffuse from the site and can initiate localized inflammatory responses by binding to specific receptors.

2. Alternate pathway

Unlike classical pathway, alternative pathway, does not require Ag-Ab complex for the initiation of complement pathway. It is initiated by cell surface constituents that are foreign to

the host. These surface molecules may be **lipopolysaccharide** etc.

When a bacterium enters the host body, as a result of inflammation, complements reach towards the site, where C3 molecules directly touch antigen and become active. In this pathway, serum C3 containing an unstable thioester bond undergoes slow spontaneous hydrolysis to yield C3a and C3b. C3b binds the surface of foreign cell and then binds to another serum protein called factor B. Now the factor B exposes the site which serves as the substrate for enzymatically active serum protein D. Then factor D cleaves B into Ba and Bb forming C3 convertase (C3bBb). C3 convertase then forms C5 convertase which ultimately forms a MAC as in classical pathway.

3. Mannose binding Lectin (MBL) Pathway

Some bacteria can activate complement system without having antibody and endotoxin. This occurs through MBL pathway which is activated when circulating lectin (MBL) binds to mannose residues on glycoproteins or carbohydrates on the surface of microorganisms. Microorganisms inducing MBL pathway are bacteria, such as Salmonella, Listeria, and Neisseria strains, some fungi and some viruses including HIV-1. MBL is an acute phase protein and its concentration increases during inflammation. The lectin recognizes and binds the carbohydrate of the target cell which then activates complements.

MBL pathway resembles classical pathway as it proceeds through the action of C4 and C2 to produce activated proteins of the complement system. MBL works same as C1q which it resembles in structure.

After the MBL binds to carbohydrate residues on the surface of a cell or pathogen, two components, MASP-1 and MASP-2 bind to MBL. MASP stands for MBL-associated serine proteases. Two proteases form a tetrameric complex similar to the one formed by C1r and C1s and cleaves C4 and C2 forming C3 convertase. The process now continues to form of C5 convertase and the MAC as in classical pathway.

Functions of Complements

Some major functions of complements are:

1. **Opsonization and phagocytosis**

C3b, bound to immune complex or coated on the surface of pathogen, activate phagocytic cells. These proteins bind to specific receptors on the phagocytic cells to get engulfed

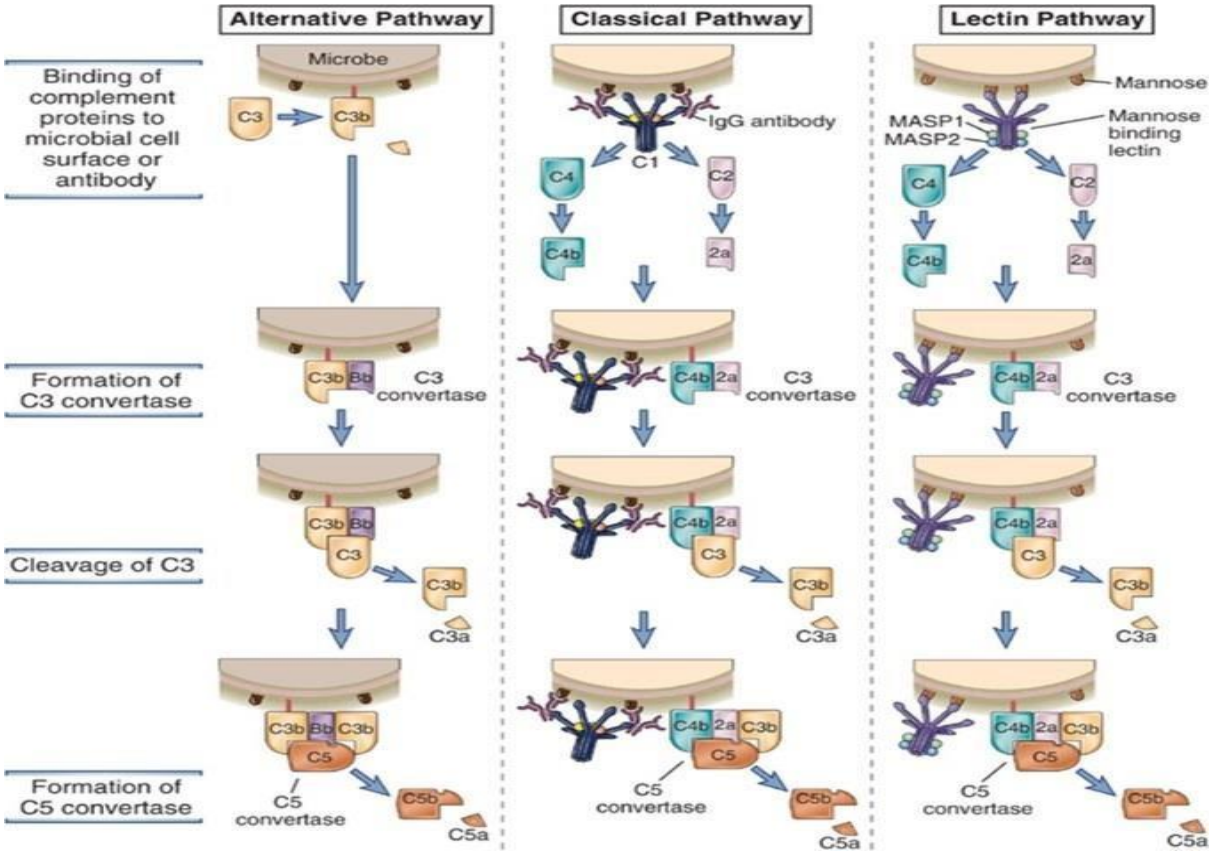


Fig. 2.7 Pathway of complement activation

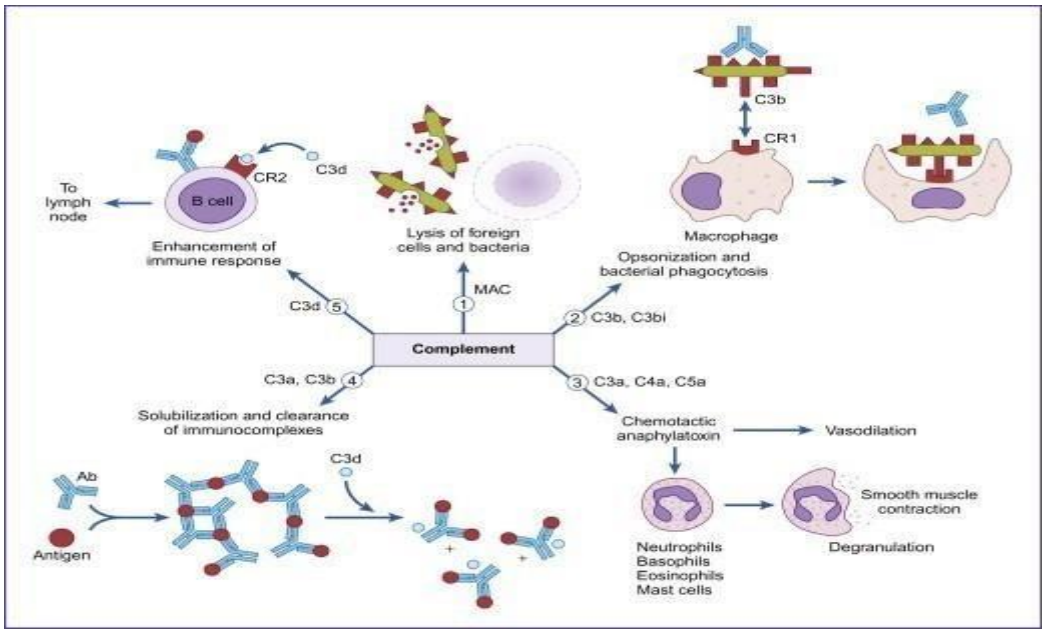


Fig. 2.8 complement activation

2. **Cell lysis**

Membrane attack complex formed by C5b6789 components ruptures the microbial cell surface which kills the cell.

3. **Chemotaxis**

Complement fragments attract neutrophils and macrophages to the area where the antigen is present. These cell surfaces have receptors for complements, like C5a, C3a, thus, run towards the site of inflammation, i.e. chemotaxis.

4. **Activation of mast cells and basophils and enhancement of inflammation**

The proteolytic complement fragments, C5a, C4a, and C3a induce acute inflammation by activating mast cells and neutrophils. All three peptides bind to mast cells and induce degranulation, with the release of vasoactive mediators such as histamine. These peptides are also called anaphylatoxins because the mast cell reactions they trigger are characteristic of anaphylaxis. Binding to specific complement receptors on cells of the immune system, they trigger specific cell functions, inflammation, and secretion of immunoregulatory molecules.

5. **Production of antibodies**

B cells have receptor for C3b. When C3b binds to B-cell, it secretes more antibodies. Thus, C3b is also an antibody producing amplifiers which converts it into an effective defense mechanism to destroy invading microorganism.

6. **Immune clearance**

The complement system removes immune complexes from the circulation and deposits them in the spleen and liver. Thus, it acts as anti-inflammatory function. Complement proteins promote the solubilization of these complexes and their clearance by phagocytes.

Complement regulation

The complement system has the potential to be extremely damaging to host tissues; hence regulatory mechanisms are required to restrict the complement pathway. Various plasma and cell membrane proteins regulate complement activation by inhibiting different steps in the cascade.

The membrane of most mammalian cells has a high level of sialic acid, which contributes to the inactivation of complements.

MONOCLONAL ANTIBODY PRODUCTION

Monoclonal antibody (MAb) is a single type of antibody that is directed against a specific antigenic determinant (epitope). It was a dream of scientists to produce MAbs for different antigens. In the early years, animals were immunized against a specific antigen, B-lymphocytes were isolated and cultured in vitro for producing MAbs. This approach was not successful since culturing normal B-lymphocytes is difficult, and the synthesis of MAb was short-lived and very limited.

It is interesting that immortal monoclonal antibody producing cells do exist in nature. They are found in the patients suffering from a disease called multiple myeloma (a cancer of B-lymphocytes). It was in 1975. George Kohler and Cesar Milstein (Nobel Prize, 1984) achieved large scale production of MAbs. They could successfully hybridize antibody— producing B-lymphocytes with myeloma cells in vitro and create a hybridoma.

The result is that the artificially immortalized B-lymphocytes can multiply indefinitely in vitro and produce MAbs. The hybridoma cells possess the growth and multiplying properties of myeloma cells but secrete antibody of B-lymphocytes. The production of monoclonal antibodies by the hybrid cells is referred to as hybridoma technology.

Principle for Creation of Hybridoma Cells:

The myeloma cells used in hybridoma technology must not be capable of synthesizing their own antibodies. The selection of hybridoma cells is based on inhibiting the nucleotide (consequently the DNA) synthesizing machinery. The mammalian cells can synthesize nucleotides by two pathways—de novo synthesis and salvage pathway (Fig. 17.1).

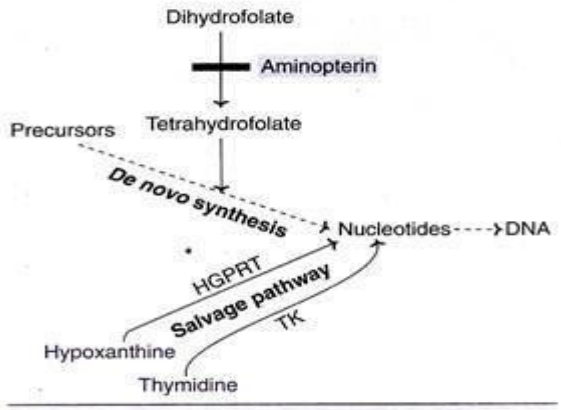


Fig. 17.1 : Pathways for the synthesis of nucleotides (HGPRT–Hypoxanthine guanine phosphoribosyl transferase; TK–Thymidine kinase)

The de novo synthesis of nucleotides requires tetrahydrofolate which is formed from dihydrofolate. The formation of tetrahydrofolate (and therefore nucleotides) can be blocked by the inhibitor aminopterin. The salvage pathway involves the direct conversion of purines and pyrimidine's into the corresponding nucleotides. Hypoxanthine guanine phosphoribosyl transferase (HGPRT) is a key enzyme in the salvage pathway of purines.

It converts hypoxanthine and guanine respectively to inosine monophosphate and guanosine monophosphate. Thymidine kinase (TK), involved in the salvage pathway of pyrimidine's converts thymidine to thymidine monophosphate (TMP). Any mutation in either one of the enzymes (HGPRT or TK) blocks the salvage pathway.

When cells deficient (mutated cells) in HGPRT are grown in a medium containing hypoxanthine aminopterin and Thymidine (HAT medium), they cannot survive due to inhibition of de novo synthesis of purine nucleotides (Note : Salvage pathway is not operative due to lack of HGPRT). Thus, cells lacking HGPRT, grown in HAT medium die.

The hybridoma cells possess the ability of myeloma cells to grow in vitro with a functional HGPRT gene obtained from lymphocytes (with which myeloma cells are fused). Thus, only the hybridoma cells can proliferate in HAT medium, and this procedure is successfully used for their selection.

Production of Monoclonal Antibodies:

The establishment of hybridomas and production of MAbs involves the following steps.

1. Immunization
2. Cell fusion
3. Selection of hybridomas
4. Screening the products
5. Cloning and propagation
6. Characterization and storage.

1. **Immunization:**

The very first step in hybridoma technology is to immunize an animal (usually a mouse), with appropriate antigen. The antigen, along with an adjuvant like Freund's complete or incomplete adjuvant is injected subcutaneously (adjuvants are non-specific potentiators of specific immune responses). The injections at multiple sites are repeated several times.

This enables increased stimulation of B-lymphocytes which are responding to the antigen. Three days prior to killing of the animal, a final dose of antigen is intravenously administered. The immune-stimulated cells for synthesis of antibodies have grown maximally by this approach. The concentration of the desired antibodies is assayed in the serum of the animal at frequent intervals during the course of immunization.

When the serum concentration of the antibodies is optimal, the animal is sacrificed. The spleen is aseptically removed and disrupted by mechanical or enzymatic methods to release the cells. The lymphocytes of the spleen are separated from the rest of the cells by density gradient centrifugation.

2. **Cell Fusion:**

The thoroughly washed lymphocytes are mixed with HGPRT defective myeloma cells. The

mixture of cells is exposed to polyethylene glycol (PEG) for a short period (a few minutes), since it is toxic. PEG is removed by washing and the cells are kept in a fresh medium. These cells are composed of a mixture of hybridomas (fused cells), free myeloma cells and free lymphocytes.

3, Selection of Hybridomas:

When the cells are cultured in HAT medium (the principle described above), only the hybridoma cells grow, while the rest will slowly disappear. This happens in 7-10 days of culture. Selection of a single antibody producing hybrid cells is very important. This is possible if the hybridomas are isolated and grown individually. The suspension of hybridoma cells is so diluted that the individual aliquots contain on an average one cell each. These cells, when grown in a regular culture medium, produce the desired antibody.

2. Screening the Products:

The hybridomas must be screened for the secretion of the antibody of desired specificity. The culture medium from each hybridoma culture is periodically tested for the desired antibody specificity. The two techniques namely ELISA and RIA are commonly used for this purpose.

In both the assays, the antibody binds to the specific antigen (usually coated to plastic plates) and the unbound antibody and other components of the medium can be washed off. Thus, the hybridoma cells producing the desired antibody can be identified by screening. The antibody secreted by the hybrid cells is referred to as monoclonal antibody.

3. Cloning and Propagation:

The single hybrid cells producing the desired antibody are isolated and cloned. Two techniques are commonly employed for cloning hybrid cells-limiting dilution method and soft agar method.

Limiting dilution method:

In this procedure, the suspension of hybridoma cells is serially diluted and the aliquots of each dilution are put into micro culture wells. The dilutions are so made that each aliquot in a well contains only a single hybrid cell. This ensures that the antibody produced is monoclonal.

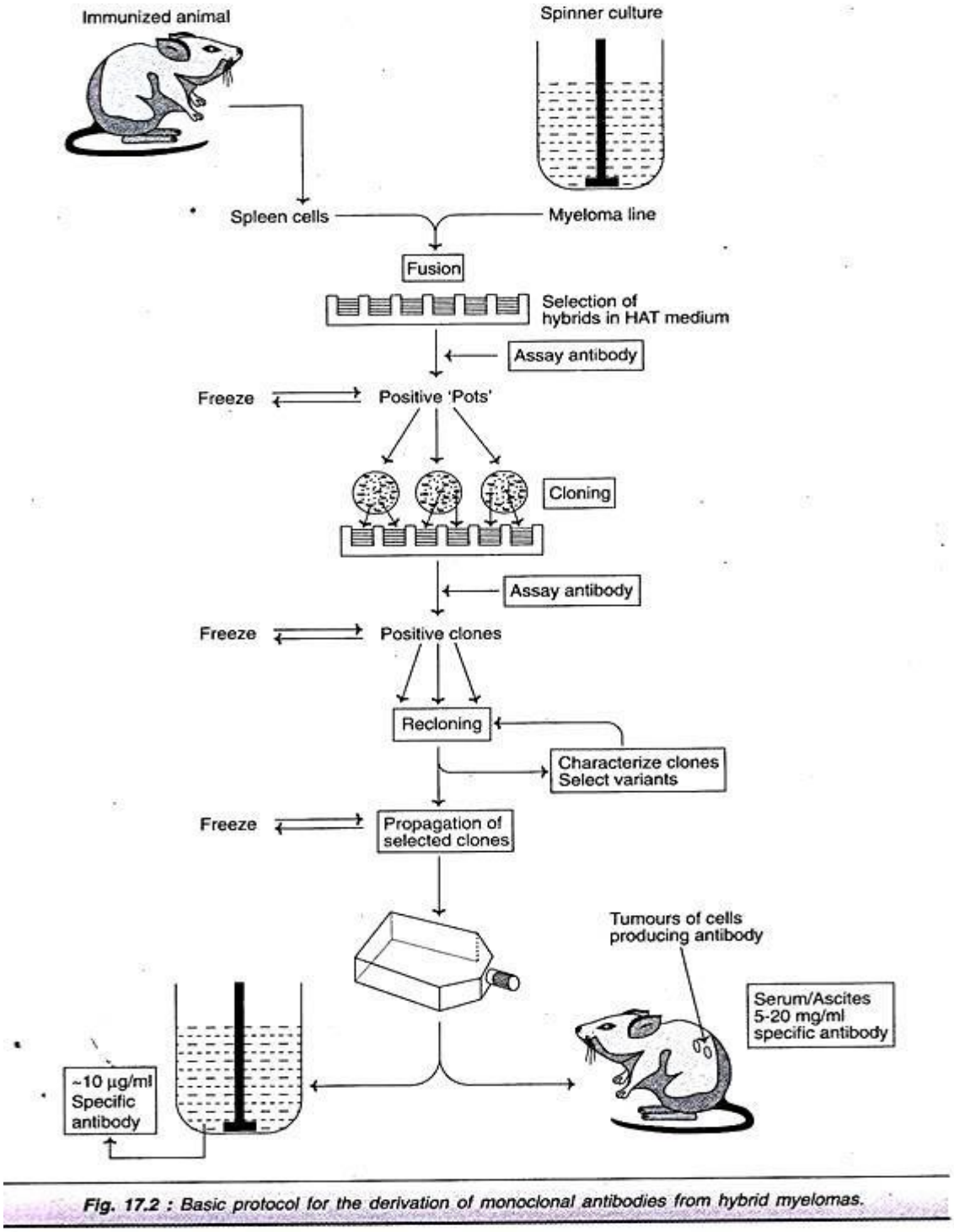


Fig. 17.2 : Basic protocol for the derivation of monoclonal antibodies from hybrid myelomas.

Soft agar method:

In this technique, the hybridoma cells are cultured in soft agar. It is possible to simultaneously grow many cells in semisolid medium to form colonies. These colonies will be monoclonal in nature. In actual practice, both the above techniques are combined and used for maximal production of MAbs.

4. Characterization and Storage:

The monoclonal antibody has to be subjected to biochemical and biophysical characterization for the desired specificity. It is also important to elucidate the MAb for the immunoglobulin class or sub-class, the epitope for which it is specific and the number of binding sites it possesses.

Large Scale Production of MAbs:

The production MAbs in the culture bottles is rather low (5-10 (ig/ml). The yield can be increased by growing the hybrid cells as ascites in the peritoneal cavity of mice. The ascitic fluid contains about 5-20 mg of MAb/ml. This is far superior than the in vitro cultivation techniques.

But collection of MAb from ascitic fluid is associated with the heavy risk of contamination by pathogenic organisms of the animal. In addition, several animals have to be sacrificed to produce MAb. Hence, many workers prefer in vitro techniques rather than the use of animals.

Procedure of Complement Fixation Test

Complement Fixation Test (CFT) consists of two stage:

First step (Complement fixation stage): a known antigen and inactivated patient's serum are incubated with a standardized, limited amount of complement.

#Note:patient's serum is heated at 56°C for 30 minutes to inactivate endogenous complement which may disturb the test calibration.

If the **serum contains specific complement activating antibody, the complement will be activated or fixed** by the antigen-antibody complex.

However, if there is **no antibody** in the patient's serum, there will be no formation of antigen-antibody complex, thus complement will not be fixed but **will remain free** (In the indicator stage this complement will react with RBC coated with antibody to sheep RBC).

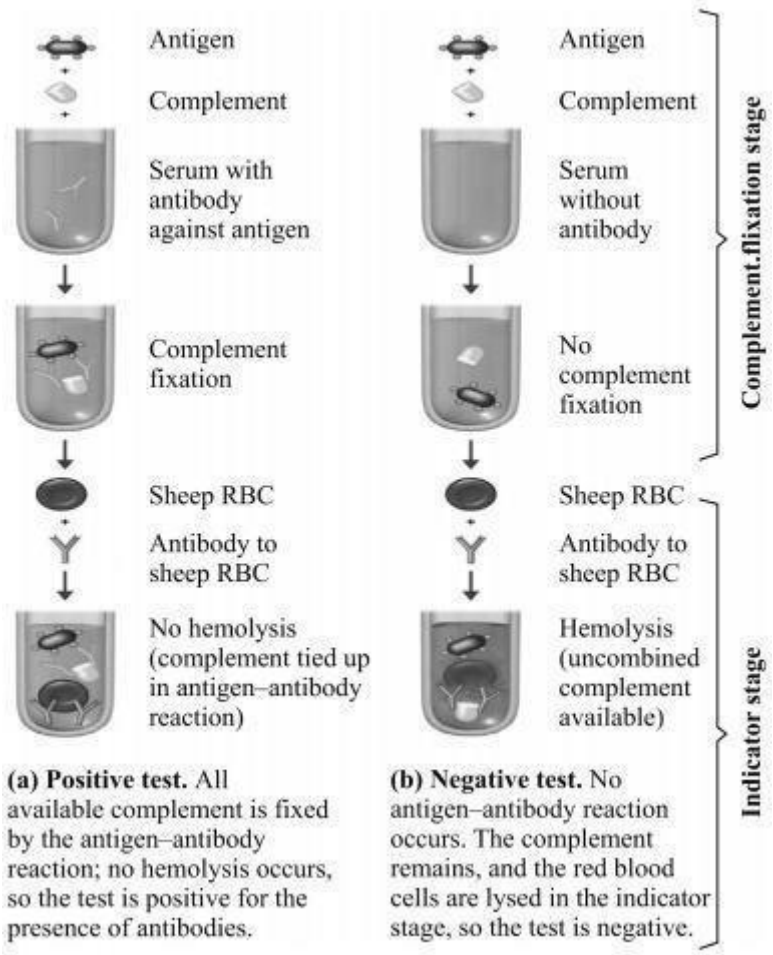


Fig.2.9 Complement fixation test

Second step (Indicator Stage): The second step detects whether complement has been utilized in the first step or not. This is done by adding the indicator system.

If the complement is fixed in the first step owing to the **presence of antibody** there will be **no complement left to fix to the indicator system**. There won't be any lysis of RBCs.

However, if there is **no** specific antibody in the patient's serum, there will be no antigen-antibody complex, therefore, **complement will be present free or unfixed in the mixture**. This unfixed complement will now react with the antibody- coated sheep RBCs to bring about their lysis.

Results and Interpretation

No lysis of sheep red blood cells (positive CFT) indicates the **presence of antibody** in the test serum,

while **lysis** of sheep red blood cells (Negative CFT) indicates the **absence of antibody** in the serum

Advantages of Complement Fixation Test

Ability to screen against a large number of viral and bacterial infections at the same time. Economical

Disadvantages of Complement Fixation Test

Not sensitive – cannot be used for immunity screening Time consuming and labor intensive

Often non-specific e.g. cross-reactivity between HSV and VZV

Vaccines- Introduction and Types with Examples

- **A vaccine** is a medical preparation given to provide immunity from a disease.
- Vaccines use a variety of different substances ranging from dead microorganisms to genetically engineered antigens to defend the body against potentially harmful microorganisms.
- Effective vaccines change the immune system by promoting the development of antibodies that can quickly and effectively attack disease-causing microorganisms when it enters the body, preventing disease development.
- A vaccine may contain live-attenuated or killed microorganisms or parts or products from them capable of stimulating a specific immune response comprised of protective antibodies and T cell immunity.
- A vaccine should stimulate a sufficient number of memory T and B lymphocytes to yield effector T cells and antibody-producing B cells from memory cells.
- The viral vaccines should also be able to stimulate high titers of neutralizing antibodies.
- Injection of a vaccine into a nonimmune subject induces active immunity against the modified pathogens.
- **Vaccination** is immunization against infectious disease through the administration of vaccines for the production of active (protective) immunity in humans or other animals.

There are 4 main types of vaccines:

1. Live Attenuated vaccines (LAV)
2. Inactivated vaccines (Killed Antigen)
3. Subunit and Conjugate Vaccines (Purified Antigen)
4. Toxoid vaccines (Inactivated Toxins)

1. Live Attenuated vaccines (LAV)

In some cases, microorganisms can be attenuated or disabled so that they lose their ability to cause significant disease (pathogenicity) but retain their capacity for transient growth within an inoculated host.

Some agents are naturally attenuated by virtue of their inability to cause disease in a given host, although they can immunize these individuals.

The first vaccine used by Jenner is of this type: vaccinia virus (cowpox) inoculation of humans confers immunity to smallpox but does not cause smallpox.

Attenuation can often be achieved by growing a pathogenic bacterium or virus for prolonged periods under abnormal culture conditions.

This selects mutants that are better suited for growth in the abnormal culture conditions than in the natural host.

For example, an attenuated strain of *Mycobacterium bovis* called **Bacillus Calmette-Guerin (BCG) Vaccine** was developed by growing *M. bovis* on a medium containing increasing concentrations of bile.

After 13 years, this strain had adapted to growth in strong bile and had become sufficiently attenuated that it was suitable as a vaccine for tuberculosis.

Due to variable effectiveness and difficulties in follow-up monitoring, BCG is not used in the United States.

The Sabin form of the polio vaccine and the measles vaccine both consist of attenuated viral strains.

Examples:

- Vaccinia (smallpox)
- Measles, mumps, rubella (MMR combined vaccine)
- Varicella (chickenpox)
- Influenza (nasal spray)
- Rotavirus
- Zoster (shingles)

2. Inactivated vaccines (Killed Antigen)

Another common means to make a pathogen safe for use in a vaccine is by treatment with heat or chemicals.

This kills the pathogen, making it incapable of replication, but still allows it to induce an immune response to at least some of the antigens contained within the organism.

It is critically important to maintain the structure of epitopes on surface antigens during inactivation.

Heat inactivation is often unsatisfactory because it causes extensive denaturation of proteins; thus, any epitopes that depend on higher orders of protein structure are likely to be altered significantly.

Chemical inactivation with formaldehyde or various alkylating agents has been successful.

The Salk polio vaccine is produced by formaldehyde inactivation of the poliovirus.

Examples:

- Polio (IPV), Hepatitis A, Rabies

3. Subunit and Conjugate Vaccines (Purified Antigen)

These subunit vaccines are composed of antigens purified from microbes which are usually administered with an adjuvant.

Vaccines composed of bacterial polysaccharide antigens are used against pneumococcus and *Haemophilus influenzae*.

Because polysaccharides are T-independent antigens, they tend to elicit low-affinity antibody responses and are poorly immunogenic in infants (who do not mount strong T cell-independent antibody responses).

High affinity antibody responses may be generated against polysaccharide antigens even in infants by coupling the polysaccharides to proteins to form conjugate vaccines.

These vaccines elicit helper T cells to simulate germinal center reactions, which would not occur with simple polysaccharide vaccines.

Such vaccines work like hapten-carrier conjugates and are a practical application of the principle of T-B cell cooperation.

Examples:

- Hepatitis B
- Influenza (injection)
- Haemophilus influenzae type b (Hib)
- Pertussis (part of DTaP combined immunization)
- Pneumococcal
- Meningococcal
- Human papillomavirus (HPV)

4. Toxoid vaccines (Inactivated Toxins)

Toxoid vaccines use a toxin (harmful product) made by the germ that causes a disease.

They create immunity to the parts of the germ that cause a disease instead of the germ itself.

That means the immune response is targeted to the toxin instead of the whole germ.

Like some other types of vaccines, you may need booster shots to get ongoing protection against diseases.

Examples:

- Diphtheria, tetanus (part of DTaP combined immunization)

Routes of immunization for injectable vaccines

In this section you will learn about the routes of administration for the injectable EPI vaccines. The injection routes are either intradermal (ID), subcutaneous (SC) or intramuscular (IM).

Intradermal immunization with BCG vaccine

Intradermal (**ID**) means within or between the layers of the skin (dermis). BCG vaccine is the only EPI vaccine which is given intradermally. BCG vaccine should be reconstituted with the appropriate diluent before administration.

Swab the outer surface of the child's outer upper right arm with antiseptic solution and allow it to dry. Select a sterile 0.1 ml syringe with a 26 gauge needle and draw 0.05 ml of the reconstituted BCG vaccine into the syringe. Hold the syringe with the needle pointing upwards and tap the syringe so any air bubbles float to the top of the barrel. Gently push the plunger just enough to expel the air through the needle — you should see a tiny drop of vaccine emerging from the tip of the needle. This ensures that you do not inject air into the child's skin. Make sure the vaccine dose is exactly 0.05 ml by checking the scale on the barrel of the syringe.

The 0.1 ml syringe and 26 gauge needle used for BCG immunizations.

Subcutaneous immunization with measles vaccine

Where vitamin A deficiency is common among children, as in Ethiopia, vitamin A drops are given at the same time as measles vaccine.

Subcutaneous (**SC**) injections are given into the fatty tissue below the skin and above the muscle. Measles vaccine is the only routine EPI vaccine which is administered subcutaneously. The vaccine comes in powder form and must be reconstituted before use with the approved diluent (as described in Section 4.1.4), and used within six hours of reconstitution.

For the immunization, select a sterile 1 ml auto-disable (AD) syringe or a sterile syringe attached to a 23-gauge needle, and draw 0.5 ml of the reconstituted measles vaccine into the syringe. Expel any air bubbles as described above for BCG immunization. Swab the skin of the child's outer upper arm with antiseptic solution and let it air dry. Hold the child's arm from below, and pinch the skin with your fingers and thumb to push up a fold of skin on top of the arm. Push the needle a little way under the pinched-up skin. The needle should go in at a sloping angle, not straight down. Inject 0.5 ml of the vaccine into the fatty subcutaneous layer below the skin, but above the muscle.

Subcutaneous immunization with yellow fever vaccine

Yellow fever vaccine is not currently used routinely in the EPI in Ethiopia, but may be required by travellers going abroad to countries where the disease is common. It is the only other

vaccine, apart from measles vaccine, which is injected subcutaneously, and is also given into the outer upper arm.

Intramuscular immunization with all other EPI vaccines

All the other EPI vaccines in routine use in Ethiopia are injected intramuscularly. **Intramuscular** (**IM**) injections are administered into the muscle layer below the skin and subcutaneous tissue, using a 1 ml syringe with a 26 gauge needle pointing straight down into the muscle.

Oral administration of OPV

OPV is a clear red or yellow liquid vaccine that may come in either of two types of containers:

- Small plastic bottles that work like droppers – the drops are given directly from the dropper into the baby's mouth.
- Glass vials with a dropper (also made of glass) supplied in a separate plastic bag. Remove the metal or plastic cap from the vial of OPV. Then cut open the plastic bag containing the dropper and fit it onto the top of the OPV vial before use.

Oral rotavirus vaccine

Rotavirus vaccine is also administered orally in a similar way as described for OPV, but Rotarix^{TM} is supplied in single-dose 'squeeze-tube' vials. Each vial contains 1.5 ml of vaccine which is squeezed slowly — drop by drop — into the infant's open mouth. You will be given more detailed instructions when the vaccine is introduced into the EPI in Ethiopia.

Table 2.1Summary of routes of administration and injection sites of the EPI vaccines.

Vaccine	**Route of administration**	**Injection site**
OPV and rotavirus vaccine (Rotarix^{TM})	Oral	None (given by mouth)
BCG	Intradermal (ID)	Outer upper right arm
Measles	Subcutaneous (SC)	Outer upper arm
Pentavalent (DPT-HepB-Hib)	Intramuscular (IM)	Outer left upper thigh
Tetanus toxoid (TT) for women of childbearing age	Intramuscular (IM)	Outer upper arm
Pneumococcal vaccine (PCV10)	Intramuscular (IM)	Outer right upper thigh

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DEPARTMENT OF BIOMEDICAL ENGINEERING

UNIT – III -Introduction to Immunology – SBMA1302

Major Histocompatibility Complex (MHC)- Types and Pathways

The major histocompatibility complex can be defined as a tightly linked cluster of genes whose products play an important role in intercellular recognition and in discrimination between self and non-self. The term 'histo' stands for tissue and 'compatibility' refers to 'getting along or agreeable'. On the other hand, the term 'complex' refers to the 'genes that are localized to a large genetic region containing multiple loci'. These genes code for antigens which involve in the determination of the compatibility of the transplanted tissue. The compatible tissues will be accepted by the immune system while the histo-incompatible ones are rejected. The rejection of foreign tissue leads to an immune response to cell surface molecules. The concept was first identified by Peter Gorer and George Snell. The main function of MHC molecules is to bring antigen to the cell surface for recognition by T cells. In humans, the genes coding for MHC molecules are found in the short arm of chromosome 6.

Genes of MHC Organized In 3 Classes

Class I MHC genes

- Glycoproteins expressed on all nucleated cells
- Major function to present processed Ags to T_C

Class II MHC genes

- Glycoproteins expressed on MF, B-cells, DCs
- Major function to present processed Ags to T_H

Class III MHC genes

- Products that include secreted proteins that have immune functions.
- Ex. Complement system, inflammatory molecules

Types

In humans, the MHC molecules are divided into three types, Class I, Class II and Class III. Class I MHC molecules are coded from three different locations called A, B and C and these molecules are expressed in all nucleated cells. Class II MHC genes are located in the D region and there are several loci such as DR, DQ and DP and these molecules are expressed only in antigen-presenting cells. Class III MHC genes are coded in the region between Class I and Class II genes. Class III MHC genes codes for cytokines and complement proteins which play an important role during the immune response.

Class I MHC molecules

- The structure of Class I MHC molecule consists of two polypeptide chains α and β. These two chains are connected together by non-covalent bonds. The α chain is characterized as an internal membrane glycoprotein with a molecular weight of 45000 Da (in humans). B chain, on the other hand, is an extracellular microglobulin with a molecular mass of 12kDa.
- The α chain is made up of approximately 350 amino acids and also divided into three globular domains α_1, α_2 and α_3. Each of these domains contains roughly 90 amino acids. The N terminal of α chain is the place of α_1 domain, while α_2 and α_3 are present after α_1 The α_2 domain is characterized by the formation of a loop of 63 amino acids; the loop is formed due to intrachain disulfide bond. α_3 also contains a disulfide bond enclosing 86 amino acids. The α_1 and α_2 domains interact to form peptide-binding units of class I MHC molecule.

- Moreover, α chain also consists of a stretch of 26 hydrophobic amino acids which holds the α chain on the plasma membrane. This transmembrane segment is present as a form of α helix at the hydrophobic region of the plasma membrane. An intracellular domain or the carboxyl-terminal of α chain is located inside the cell and it contains around 30-40 amino acids.
- In humans the β chain is non-polymorphic and it is dimorphic in mice. α_3 and β chain are structurally similar to the immunoglobulin C domain and also characterized as a disulfide loop. A peptide binding platform is formed by β plated sheets of α_1 and α_2
- T_{cyt} Cell (cytotoxic T cell) has specificity towards cells containing peptides associated with Class I MHC due to the presence of CD8 antigen on the surface of Tcyt Cell. CD8 antigen has an affinity towards the α_3 domain of Class I MHC molecules.

Class II MHC molecules

- Class II MHC molecules are heterodimers and characterized by two non covalently connected polypeptide chains. The chains are termed as a heavy chain (α, 30kDa) and light chain (β, 26kDa).
- Similar to class I MHC molecules, class II MHC molecules are also characterized by an extracellular amino terminal domain, a transmembrane domain and an intracellular carboxy terminal tail.
- The class II MHC molecules are expressed on the surface of the antigen-presenting cells such as B cells, dendritic cells, and macrophages.
- The α chain is divided into two domains α_1 and α_2, while the β chain is also divided into two groups β_1 and β_2. The β_2 domain is responsible for the binding of T cell co-receptor CD4. The α_1 and β_1 domain, on the other hand, involved in the formation of the antigen-binding sites. Peptides containing 13-20 amino acids can bind at the antigen-binding site of class II MHC.
- The presence of disulfide bonds in α_2, β_1, and β_2 domains are also an important structural feature of the class II MHC molecules.

Class III MHC molecules

- There are several serum proteases which involve in compliment system come under the group of class III MHC molecules.
- Class III MHC molecules do not have any involvement in antigen presentation.
- The complement components such as asC2, C4A, and C4B, and factor B are the most important compounds involve as class III MHC molecules. Apart from these tumor necrosis factors α and β and some heat shock proteins also come under this category.

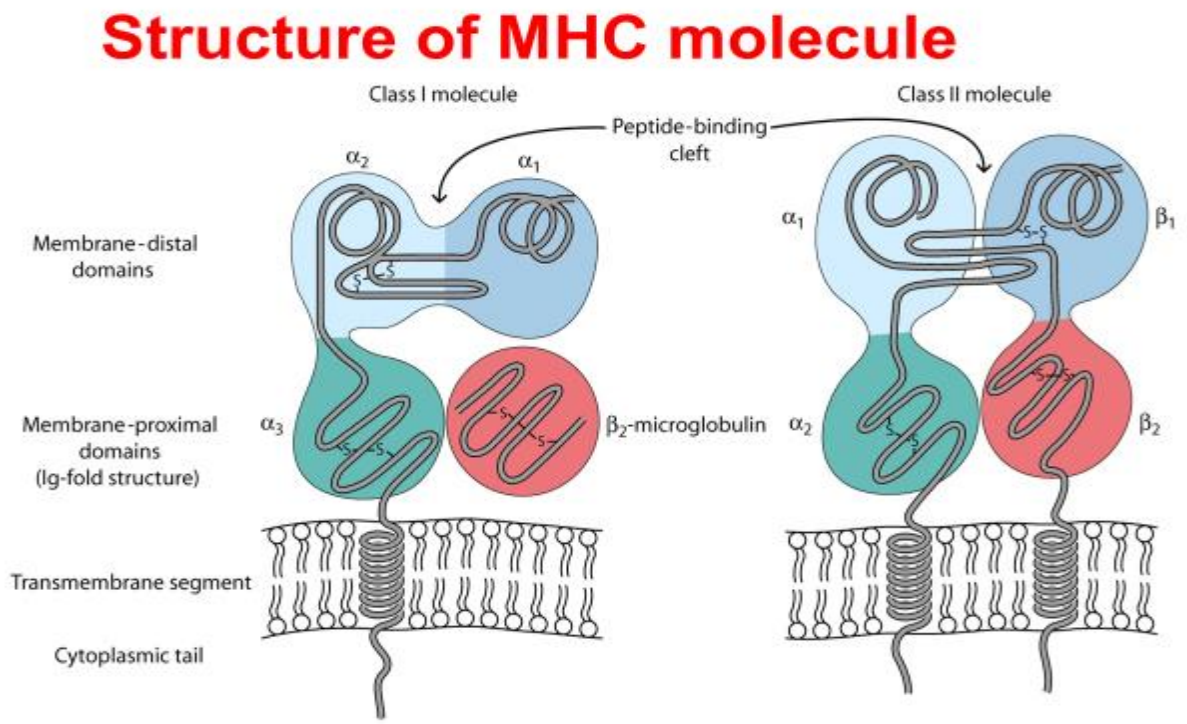

Fig. 3.1 Structure of MHC molecules

MHC polymorphism and co-dominant expression

An individual has two HLA-A genes, one on the paternal, one on the maternal chromosome 6. But, looking at the human population, there are more than 4700 gene variants of HLA-A, many of which (more than 3200) lead to small differences in the encoded protein. Most of these differences cluster around the binding cleft, modifying its preference for specific peptides. In other words, HLA-A is polymorphic. Our maternal and paternal alleles are unlikely to be identical, and we are unlikely to share identical HLA-A-alleles with an unrelated individual. The same is true for all other HLA loci with exception of HLA-DRα, which is identical in nearly all of us. Both of the two alleles are expressed at the same time in the same cell. As both contribute to the phenotype and none dominates over the other, we call this co-dominant expression. Individual genetic alleles are designated with a locus-asterisk-number combination (e. g., A*01:01:01:01 and A*02:86:01:01). The first group of digits following the asterisk try to reflect the antibody-determined serotype, the next two or three digits indicate subtypes characterized by differences in amino acids, and further groups of digits reflect differences that are present only at the DNA, but not at the protein level (silent and intron polymorphisms, respectively). The combination of polygeny of the individual (9 gene loci: A, B, C, DRα, DRβ, DQα, DQβ, DPα, DPβ) with polymorphism in the population (for 8 out of these 9 loci) means it is extremely unlikely to find two unrelated individuals with exactly the same MHC. 33 This causes problems in organ transplant. A vigorous immune response is mounted against HLA molecules unknown to the immune system by both cytotoxic T cells and antibodies. From this perspective, polymorphism of MHC seems rather undesirable. As evolution resulted in this extreme form of polymorphism, it has to involve some selectable advantage. Most likely, the variation in MHC alleles allows at least part of the population to successfully fight any

epidemic infection. Think of the plague in medieval Europe around AD 1350: had everybody been equipped with the same MHC, possibly no one would have survived.

Antigen processing and presentation

The T cells can recognize the foreign antigen when the antigen is attached to the MHC molecules as an MHC peptide complex. The formation of the MHC-peptide complex requires the degradation of protein antigen by several steps. The degradation process is known as antigen processing. These degraded proteins are then attached to the MHC molecules inside the cell and then the MHC molecules transported to the membrane to present the antigen with the T cell.

Antigen processing and presentation: Class I MHC molecules (Cytosolic pathway)

- Class I MHC molecules involve in presenting intracellular or endogenous pathogens or antigens. Intracellular pathogens refer to those organisms which live and replicates inside the host cell. An example of this type of pathogen is a virus.
- Under normal condition the MHC class I molecules forms a complex with the self-peptides or self-antigens. While, in case of any viral infection, the MHC class I molecules present the peptide derived from the virus which is then further recognized by T cells.
- Cell components such as a nucleus, endoplasmic reticulum and Golgi apparatus play an important role in antigen processing and presentation.
- When a virus infected a normal cell, the viral DNA moves inside the cell and produce viral proteins with the help of the host cell mechanisms. The viral proteins are synthesized in the cytosol.
- The cytoplasm also contains a cylindrical protein complex called the proteasome. The main function of the proteosome is to degrade the unwanted or damaged protein into smaller peptides. At the time of viral infection, the viral proteins interacted with the proteosomes present in the cytoplasm. The processing took place in the cytosol and as a result, the proteins are degraded into smaller peptides (8-15 amino acid long).
- In the next step, these fragmented peptides are transported into the endoplasmic reticulum. The transport took place due to a peptide delivery system called the transporter associated with antigen processing (TAP). TAP is made up of two domains or subunits called TAP 1 and TAP 2.
- Inside the endoplasmic reticulum the α and β chains of MHC class I molecules are synthesized and by the help of a group of chaperone proteins, the MHC class I molecule is formed and moves towards the TAP. As a result, the peptides bind at the peptide-binding site of the class I MHC molecule inside the endoplasmic reticulum and forms the MHC class I-peptide complex.
- In the next step, the MHC class I- peptide complex moves to the surface of the Golgi apparatus and by the help of secretory vesicle, it moves towards the surface of the plasma membrane.
- Once the MHC class I-peptide complex reaches the cell surface, the T cell receptors recognize the antigen peptide complex. Moreover, the co-receptor CD8 of the T cell attaches with the α_3 domain of the MHC class I molecule. Hence, the antigen is presented to the T cell.

Antigen processing and presentation: Class II MHC molecules (Endocytic pathway)

- MHC class II molecules are responsible for presenting exogenous or extracellular pathogen or antigen. The extracellular pathogen refers to the organisms which can grow and reproduce outside of the host cell. Bacteria, exotoxins, parasites are examples of extracellular antigens. These antigens are taken up by the cell by endocytosis or phagocytosis.
- Only the antigen-presenting cells involved in antigen processing and presentation by MHC class II molecules. These cells include B cells, macrophages, and dendritic cells. The pathway took place only after the engulfment of the antigen by the antigen-presenting cells.
- Inside the cell, the antigen carries a covering called an endosome. The endosome is fused with the lysosome present in the cytoplasm and forms endolysosomes. As a result, the foreign protein is degraded by the proteolytic enzyme present inside the lysosome and small peptides are formed.
- The class II MHC molecules are synthesized and formed in the endoplasmic reticulum. The α and β chain of the molecule is also associated with the invariant chain. This association helps to restrict the binding of self-antigen with the class II MHC molecule. The invariant chain- MHC complex is then transported from the endoplasmic reticulum to the Golgi apparatus and from the Golgi apparatus to another vesicle. Inside the vesicle, the invariant chain is digested and only a small fragment (Class II-associated invariant chain polypeptide: CLIP) is attached with the molecule.
- In the next step, the vesicle containing the MHC class II molecule is then fused with the vesicle containing fragmented peptides. The fragmented peptide is then bound with the MHC class II molecule by displacing the CLIP. This newly formed MHC class II-peptide complex is then transported to the surface of the cell.
- Once at the cell surface, the antigen is presented to the T cells. The T cell recognizes the peptide bound with the MHC class II molecule by the help of the T cell receptor and the CD4 co-receptor binds with the β_2 domain of the class II MHC molecule.

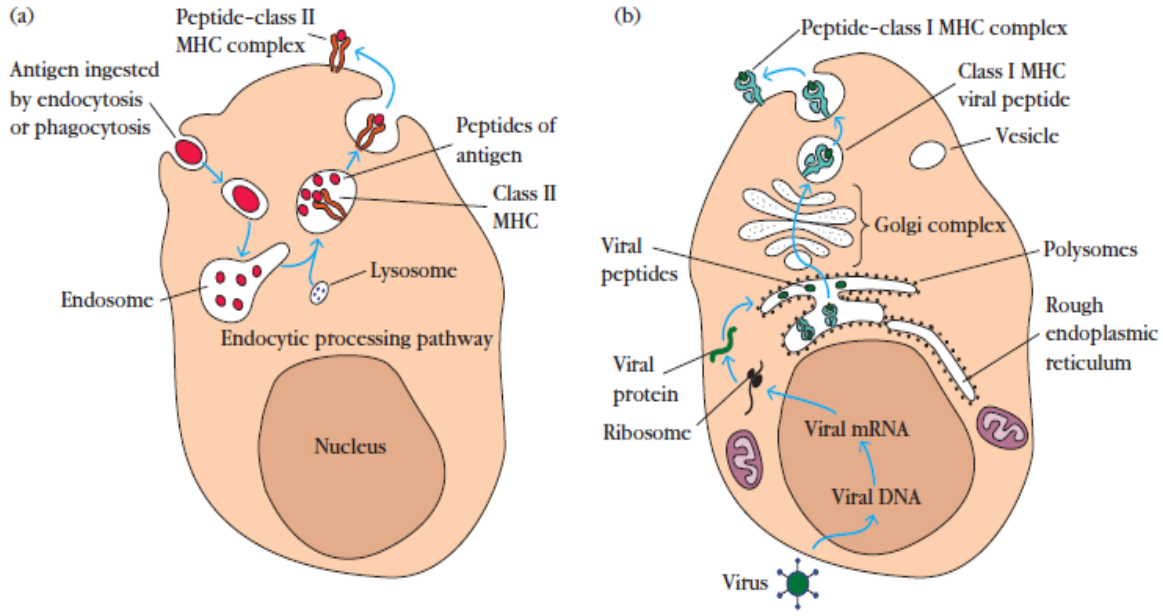

Fig. 3.2 Antigen processing and presentation (Endocytic and exocytic pathway)

MHC class II polymorphism

Like the MHC class I heavy chain, human MHC class II molecules are encoded by three polymorphic genes: HLA-DR, HLA-DQ and HLA-DP. Different MHC class II alleles can be used as genetic markers for several autoimmune diseases, possibly owing to the peptides that they present.

Phagocytosis

Phagocytosis is a type of endocytosis whereby a cell engulfs a particle to form an internal compartment called a **phagosome.** The cell rearranges its membrane to surround the particle that is to be phagocytosed and internalises it.

Within the phagosome that then forms the particle can be degraded. In the immune system, it is a major mechanism that the body uses to remove potentially **pathogenic material.**

Phagocytic Cells of The Immune System

Many cells are capable of phagocytosis, but several types of immune cells are particularly specialised in this role:

Neutrophils – these are abundant in the blood and important in acute inflammation, as they are the first immune cells to arrive at the site of infection

Macrophages – tissue resident cells that are key as a first defence mechanism and in initiating the adaptive immune response

Dendritic cells – these cycle through the bloodstream, tissues and lymphoid organs, sampling potential pathogens and acting as a major link between the innate and adaptive immune systems.

Stages of Phagocytosis

Activation

Resting phagocytes become activated by inflammatory mediators (e.g. bacterial proteins, capsules, peptidoglycan, prostaglandins, complement proteins). The result is that they gain the ability to leave the capillaries and enter the tissues towards the site of infection **(chemotaxis).**

Phagocytes switch to a higher energy level. This usually involves rearrangement of the cell **cytoskeleton** and swelling of the cell (caused by calcium and sodium ion influx).

Phagocytes also produce **pattern recognition receptors** (PRRs) which recognise and bind to pathogen-associated molecular patterns (PAMPs). PAMPs are components of pathogens and can include molecules like peptidoglycan and lipopolysaccharide (LPS).

Chemotaxis

This is the directional movement of the phagocyte towards a chemical attractant (chemotaxin). **Chemotaxins** include bacterial products (e.g. endotoxin), injured tissues, complement proteins (C3a, C4a, C5a) and chemical substances produced by leukocytes (leukotrienes).

The process of chemotaxis is usually coupled with activation.

After attachment, the phagocyte internalises the microbe into a phagosome. The phagosome then fuses with a lysosome to form a **phagolysosome**. Lysosomes contain digestive enzymes which can destroy the internalised material.

Ingestion of Antigens

Endocytosis is a general term describing a process by which cells absorb external material by engulfing it with the cell membrane. Endocytosis is usually subdivided into pinocytosis and phagocytosis. If the antigen is a small-molecular-weight protein or a polysaccharide, the cell membrane invaginates in a process called *pinocytosis*, and the protein or carbohydrate is placed in a fluid-filled sack called a *vesicle*. Large-molecular-weight antigens or intact microbes are internalized by a different form of endocytosis, which is called *phagocytosis*. During phagocytosis, the membrane envelopes the particle to form an internal vacuole termed *phagosome*.

Intracellular Killing of Bacteria

In the phagosome, bacteria are killed by two different mechanisms. One mechanism is dependent on the presence of oxygen, a respiratory burst, and the generation of reactive oxygen species. The second mechanism (which is independent of oxygen) uses preformed granules containing proteolytic enzymes to kill microbes.
Pathogen killing can occur in one of two ways:

- The **oxygen dependent pathway** (oxidative burst) involves the generation of reactive oxygen species (ROS) such as the superoxide radical and hydrogen peroxide. These highly reactive radical molecules react with proteins, lipids and other biological molecules to kill the pathogen.

  Superoxide radicals are initially produced (via the enzyme NADPH oxidase) and subsequently converted to hydrogen peroxide by the enzyme **superoxide dismutase**.

  Superoxide radicals can also react with the hydrogen peroxide to form powerful hydroxyl radicals which assist in killing the invading pathogen.

  Oxygen-Dependent Respiratory Burst

  Following the ingestion of microbes, an oxygen-dependent respiratory burst occurs, with rapid production of singlet oxygen ($O-2O_2^-$) and hydrogen peroxide (H_2O_2) and energy in the form of adenosine triphosphate (ATP). In the oxidative phosphorylation pathway, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase generates singlet oxygen as it transfers electrons to the cytochrome system.

  2O2+ NADPH2O2−+ NADP++ H+

- The **oxygen independent pathway** involves the destruction of the pathogen via lysosomal enzymes such as proteases, phospholipases, nucleases and lysozyme. These enzymes help in destroying the pathogen primarily by breaking down its cell membrane. This a less effective mechanism when compared to the oxygen dependent pathway.

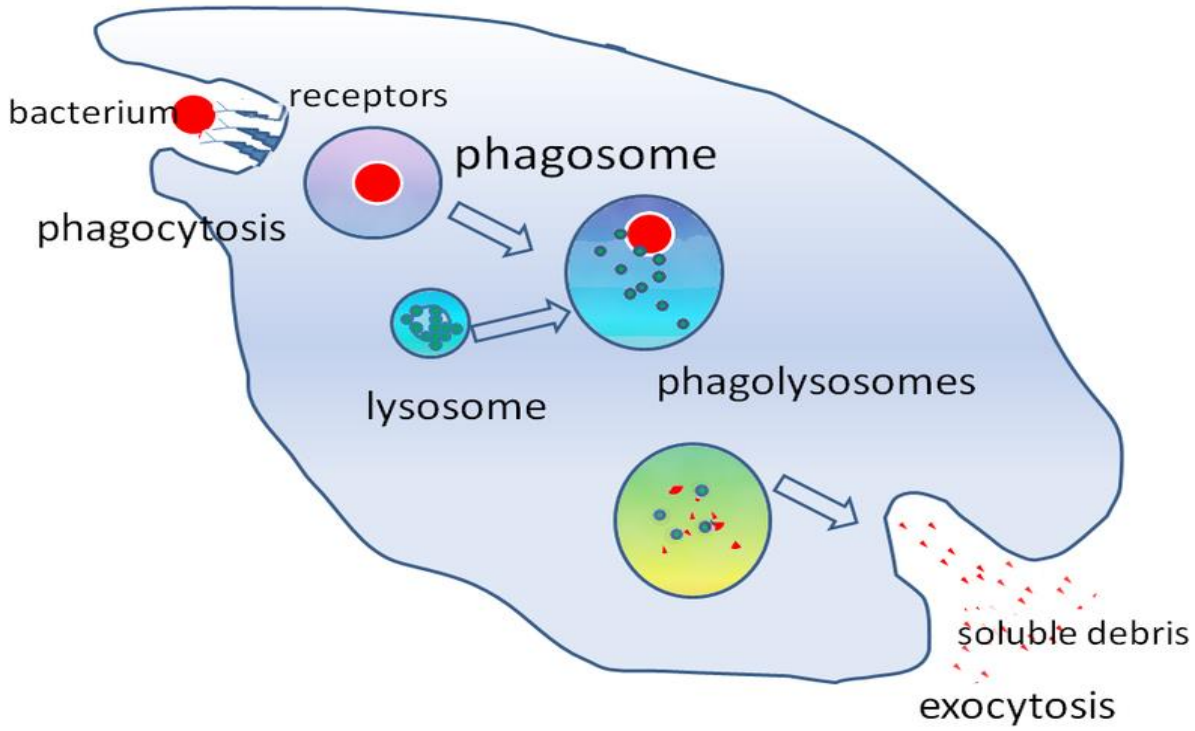

Fig. 3.3 Steps involved in phagocytosis

Cytokines

Definition, Types, Receptors, Functions

cytokines are a variety of regulatory/signaling molecules (small proteins or glycoprotein) produced by various nucleated cells in the body. As products of nucleated cells (various cells of the immune system), they play a crucial role in the regulation of various aspects of immunity including the intensity and duration of immune responses by immune cells, etc.

Based on studies, cytokines have also been shown to be pleiotropic and therefore have varying impacts on different types of cells. However, different types can also influence the activities of others and vice versa.

In addition to mediating and regulating immune responses, cytokines are also involved in the production and development of all the different types of blood cells (through a process known as haematopoiesis), angiogenesis as well as tumorigenesis among a few other important functions.

Types of Cytokines

- Interferons
- Chemokines
- Interleukins (many interleukins are considered to be lymphokines)
- TNF (tumor necrosis factor)

Functions

Interleukins

Produced by such leukocytes as lymphocytes and monocytes (and a number of other cells in the body e.g.) interleukins are glycoproteins involved in the activation and differentiation of immune cells. In addition, they also play an important role in the proliferation, migration, maturation, pro- and anti-inflammatory activities, as well as adhesion of these cells.

Together with interleukin receptors, interleukins belong to a superfamily (IL superfamily) that is made up of proteins. While it's easy to generalize the functions of these cytokines, this is largely dependent on the type of interleukins. Currently, over 43 members of this superfamily have been identified (IL-1 to IL-43).

Like some of the other cytokines, interleukins are made up of proteins. Typically, this is in response to invading pathogens/antigens. While high volumes may be produced depending on the type of interleukins and the invading organism, a small quantity of the molecule is required to activate biological effects.

While a good number of interleukins elicit an action on the same cells that produced them, some can enter the bloodstream which allows them to be transported and elicit biological effects on distant cells in the body. For instance, while IL-2 elicits biological effects on T cells (which produced them), IL-1 can enter the bloodstream and reach the central nervous system.

Some of the other properties of interleukins include:

- Synthesis is a self-limited process
- Stimulate up-regulatory and down-regulatory mechanisms
- Have redundant functions in the body - Given interleukins can cause different effects on various types of cells
- Can influence the synthesis and functioning of other interleukins
- As already mentioned, different types of interleukins have different functions. The following are some of the functions of a few of these molecules:

IL-1 - Indirectly stimulates immune responses via various effector proteins and other cytokines. IL- - Interleukin 2 plays an important role in growth regulation of T cells.

IL-3 - Stimulates the production of myeloid progenitor cells by hematopoietic stem cells. However, an interaction between IL-3 and IL-7 results in the production of lymphoid progenitor cells from hematopoietic stem cells.

IL-4 - Interleukin 4 is involved in a number of biological processes ranging from the proliferation of T cells and B cell stimulation to humoral and adaptive immunity. Moreover, it

contributes to the production of a number of cells including dendritic cells, Th1 cells as well as Interferon Gamma cells.

IL-5 - Like Il-4 and IL-13, interleukin 5 is also involved in stimulating the growth of B-cells. However, it is also involved in increased secretion of immunoglobin as well as activation of eosinophils.

IL-6 - In addition to being the primary mediator in such illnesses as fever, interleukin 6 has also been shown to overcome the blood-brain barrier in activating the expression of Prostaglandin E2 in the hypothalamus which results in body temperature change.

Some of the other common interleukins include:

- IL-8 - involved in the induction of chemotaxis
- IL-10 - involved in inflammation as well as regulation of the immune responses
- IL-18 - involved in both immune responses in the body (innate and adaptive responses)
- IL-33 - involved in immune responses of Th2 (e.g. parasitic infections)

Interferons (IFNs)

Interferon is a family consisting of widely expressed signaling proteins. Like the other cytokines, interferons are also released by cells of the host's immune system in response to such invading organisms as bacteria and viruses.

They are also released to respond to tumorous cells in some organisms. Currently, three types of interferons have been identified. These include Type I IFNs, Type II IFN, and Type III IFNs.

Type I IFNs (Type I Interferons)

Type I interferons are divided into two major groups that include IFN-α and IFN-β as well as a number of additional isotypes that include, among others, IFN-κ, IFN-ω, and IFN-δ. While only one type of IFN-β exists, IFN-α is further divided into several subtypes including IFN-α1, IFN-α2, IFN-α3, IFN-α4, IFN-α5, and IFN-α6 among others.

In the body, the production of Type I IFNs is dependent on the presence of various microorganisms. For instance, following a viral infection, a signaling pathway that causes phosphorylation, dimerization as well as passage of the interferon response factor 3 (IRF3) to the nucleus is activated.

Along with a number of other transcription factors, IRF3 activates that synthesis of IFN-β gene that binds to the interferon receptors located on the surface of an infected cell which ultimately results in interferon response.

Through these responses, interferons help in the recruitment of effector molecules that protect the cells against infections (bacterial and viral infections). For instance, by activating the

production of natural killer cells and macrophages, interferons contribute to the destruction of both the viruses and infected cells.

***** Cells responsible for the production of IFN-α and IFN-β are collectively known as interferon-producing cells (IPCs) or natural interferon-producing cells.

IFN production may be presented as follows:

Functions of Type I interferons (IFN-α and IFN-β) are generally divided into three main categories that include:

・Influence resistance to viral replication in cells - This is achieved through the destruction of viral mRNA required for viral replication as well as inhibiting the translation of viral proteins.

・Promote ligand increase - Type I interferons promote an increase in ligands to the receptors of NK cells which in turn stimulates them to attack and lyse infected cells.

・Activate the destruction of infected cells by NK cells and macrophages.

***** Given that Type 1 interferons also plan a role in immunosuppressive activities, they are also used for the purposes of treating autoimmune diseases.

Type II Interferon

Type II interferon is made up of a single cytokine known as IFN-y. This cytokine is largely produced by THI T cells, activated Natural Killer cells as well as CD8+ T cells. Unlike cytokines of Type I interferons, the gene responsible for encoding the Type II cytokine is located in chromosome 12 in human beings.

Moreover, IFN-y has been shown to be different from the other interferons in that it does not produce a potent antiviral effect. Rather, it largely serves to activate effector cells.

While it's produced by T cells in adaptive immunity (following an increase in antigen), IFN-y is produced by natural killer cells in the innate immune system and acts as a mediator. In the adaptive immune system, increased production of IFN-y is promoted by IL-12 and IL-18.

On the other hand, IL-4 and IL-10 correspond to the negative regulators involved in its production. Apart from T cells, B cells and professional antigen-presenting cells have also been shown to play a role in the production of IFN-y.

Like cytokines of Type I interferons, IFN-y also contributes to cell response to viral infections. For instance, by activating and causing the induction of MHC (major histocompatibility complex), IFN-y has been shown to play a role in long-term control of viral infections in cells. In the process, it also coordinates the transition from innate to adaptive immunity.

Some of the other functions of IFN-y include:

· Macrophage activation - By promoting the activation of macrophages, IFN-y contributes to phagocytic and pinocytic activities of these cells and thus contributes to microbial destruction.

· Inhibiting cell growth - In the body, IFN-y has also been shown to inhibit cell growth and thus promote apoptosis.

Type III interferons

Unlike Type II interferon that only consists of a single cytokine, Type III is divided into three important cytokines that include IFN-λ1, IFN-λ2, and IFN-λ3. Also known as IL-28 (a and b) and IL-29, Type III interferons are characterized by a structure that is more similar to proteins of the family IL-10.

On the other hand, the signaling pathway of these cytokines has been shown to resemble that of Type I interferons in that they are dependent on the actions of IRFs and NF-kB. While these cytokines also regulate a number of similar functions as Type I interferons, they primarily function in mucosal epithelial cells (as well as liver cells in human beings) where they serve to protect them from viral infections.

Chemokines

Chemokines are a type of protein cytokine that play an important role in chemotaxis. As such, there may be signals that guide certain immune cells to the affected site.

Currently, about 50 chemokines have been identified. They are divided into four families based on the location of N-terminal cysteine residues in their three-dimensional structure.

Tumor Necrosis Factor

Tumor Necrosis Factor (TNF) consists of a group of proteins involved in a number of physiological and pathological processes. Currently, about 40 members of the superfamily (TNF) have been identified with TNF-α and TNF-β being the most notable examples.

TNF-alpha (TNF-α)

Also known as TNF or TNFSF2, TNF-α is a multifunctional cytokine involved in such processes as apoptosis and coagulation among others. In human beings, TNF-α genes are located on chromosome 6. This allows the cytokine to be expressed and produced by such immune cells as macrophages, monocytes and T cells in response to invading pathogens or the presence of cytokines like IFN-y.

Some of the main characteristics of TNF-α include:

· Following activation of macrophages and other cells, TNF-α is synthesized as a 26-kD nonglycosylated type II membrane protein.

· Two types of TNFα that include mTNFα and sTNFα.

· Due to adipose tissue expression, TNF-α is sometimes referred to as an adipostat.

Functions of TNF-α

As mentioned, TNF-α is a multifunctional cytokine that ranges from immune roles to programmed cell death.

Immune function - Like the other cytokines, TNF-α plays an important role in immunity. In particular, TNF-α attracts certain immune cells to the affected site by stimulating the expression of adhesion molecules by vascular endothelial cells. This makes it possible for immune cells to adhere to blood vessel walls and successfully migrate to the infected site and destroy invading pathogens (bacteria and viruses).

Induces the production of chemokines that are involved in inflammatory responses - These cytokines guide immune cells to the affected site.

Apoptosis - TNF-α promotes the programmed cell death of tumor cells by promoting the recruitment of proteins involved in death signaling.

Biological functions - When produced in large amounts, TNF-α has been shown to induce reduced blood pressure or shock during such events as severe infections. In some cases, however, a high concentration of this cytokine results in low blood sugar concentration as well as intravascular thrombosis.

TNFβ - Also known as Lymphotoxin, TNFβ is a type II transmembrane protein. The expression of this cytokine is stimulated by activated lymphocytes. With regards to functions, TNFβ is a potent mediator involved in various immune and inflammatory responses that have similarities to those of TNF-α.

Like TNF-α, TNF-β is also involved in the following processes:

- Apoptosis
- Coagulation
- Cell proliferation and differentiation

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UNIT – IV – Introduction to Immunology – SBMA1302

IMMUNOTECHNOLOGY

Antigen-Antibody Reactions

The interactions between antigens and antibodies are known as ***antigen–antibody reactions***. The reactions are highly specific, and an antigen reacts only with antibodies produced by itself or with closely related antigens. Antibodies recognize molecular shapes (epitopes) on antigens. Generally, the better the fit of the epitope (in terms of geometry and chemical character) to the antibody combining site, the more favourable the interactions that will be formed between the antibody and antigen and the higher the affinity of the antibody for antigen. The affinity of the antibody for the antigen is one of the most important factors in determining antibody efficacy *in vivo.*

Agglutination

Agglutination reactions involve particulate antigens capable of binding antibody molecules. Since antibody molecules are multivalent, suspended particulate antigens form large clumps or aggregates, easily visible without magnification, when exposed to specific antibodies. Antibodies that cause this reaction are referred to as agglutinins. Agglutination assays can be used to determine concentrations of specific antibodies in a patient's immune sera. A constant amount of a suspended particulate antigen is added to a series of tubes containing a twofold dilution of patient's immune serum, and the titer of antibody in the serum is the reciprocal of the highest serum dilution showing agglutination of the particulate antigen. Agglutination reactions are routinely used for identification and serotyping of a wide range of bacterial foodborne pathogens.

Blood Cell Antigens and Antibodies

Agglutination of red cells by antibody: a basic method

Agglutination tests are usually carried out in tubes, microtitre plates or using column agglutination (gel) technology, centrifugation or sedimentation. Rarely slide tests are used for emergency ABO and D grouping. For microplate tests, Tube tests.Add 1 volume of a 2% red cell suspension to 2–3 volumes of plasma in a disposable plastic tube. Mix well and leave undisturbed for the appropriate time (see below).Tubes.For agglutination tests, use medium-sized (75 × 10 or 12 mm) disposable plastic tubes. Similar tubes should be used for lysis tests when it is essential to have a relatively deep layer of serum to look through, if small amounts of lysis are to be detected. The level of the fluid must rise much higher than the concave bottom of the tubes.Glass tubes should always be used if the contents are to be heated to 50 °C or higher or if organic solvents are being used. Glass tubes, however, are difficult to clean satisfactorily,

particularly small-bore tubes and cleaning methods such as those given on p. 564 should be followed carefully.Temperature and time of exposure of red cells to antibody

In blood group serology, tube tests are generally done at 37 °C, room temperature or both. There is some advantage in using a 20 °C waterbath rather than relying on 'room temperature', which in different countries and seasons may vary from 15 °C (or less) to 30 °C (or more).Sedimentation tube tests are usually read after 1–2 h have elapsed. Strong agglutination will, however, be obvious much sooner than this. In spin-tube tests, agglutination can be read after only 5–10 min incubation if the cell–plasma mixture is centrifuged.

Slide tests

These are used rarely in a few parts of the world. Because of evaporation, slide tests must be read within about 5 min. Reagents that produce strong agglutination within 1–2 min are normally used for rapid ABO and RhD grouping. Because the results are read macroscopically, strong cell suspensions should be used (35–45% cells in their own serum or plasma).

Reading results of tube tests

Only the strongest complete (C) grade of agglutination seems to be able to withstand a shake procedure without some degree of disruption, which may downgrade the strength of reaction. The British Committee for Standards in Haematology (BCSH) Blood Transfusion Task Force has therefore recommended the following reading procedure.30

Microscopic reading.

It is essential that a careful and standardised technique be followed. Lift the tube carefully from its rack without disturbing the button of sedimented cells. Holding the tube vertically, introduce a straight-tipped Pasteur pipette. Carefully draw up a column of supernatant about 1 cm in length and then, without introducing an air bubble, draw up a 1–2 mm column of red cells by placing the tip of the pipette in the button of red cells. Gently expel the supernatant and cells onto a slide over an area of about 2 × 1 cm. It is important not to overload the suspension with cells and the method described earlier achieves this.

Macroscopic reading

A gentle agitation tip-and-roll 'macroscopic' method is recommended. It is possible to read agglutination tests macroscopically with the aid of a hand reading glass or concave mirror, but it is then difficult to distinguish reactions weaker than + (microscopic reading) from the normal slight granular appearance of unagglutinated red cells in suspension. Macroscopic reading thus gives lower titration values than does microscopic reading, but the former is recommended. Follow the system of scoring in Table 21-11.

A good idea of the presence or absence of agglutination can often be obtained by inspection of the deposit of sedimented cells: a perfectly smooth round button suggests no agglutination, whereas agglutination is shown by varying degrees of irregularity, ‘graininess’, or dispersion of the deposit.

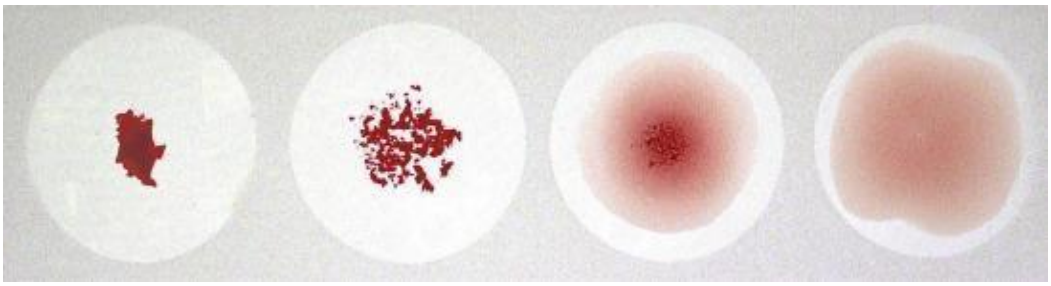

Fig. 4.1 Blood agglutination

Agglutination is shown by various degrees of ‘graininess’; in the absence of agglutination, the sedimented cells appear as a smooth round button, as on the extreme right.

Toxoplasma gondii Agglutination and AC/HS Tests

The agglutination test using formalin-preserved whole tachyzoites is available commercially (bioMérieux, Marcy-l'Etoile, France) and detects IgG antibody. The test is very sensitive to IgM antibody, and “natural” IgM antibody causes nonspecific agglutination in sera that yield negative results when tested in the DT and the IFA test. This problem is avoided by including 2-mercaptoethanol in the test. The method is accurate, simple to perform, inexpensive, and excellent for screening purposes.307 This method, that is, with mercaptoethanol, should not be used for the measurement of IgM antibodies.

When two different compounds (i.e., acetone and formalin) are used to fix parasites for use in the agglutination test, a “differential” agglutination test (AC/HS test) results because the different antigenic preparations vary in their ability to recognize sera obtained during the acute and chronic stages of the infection.308 This test has proved useful in helping differentiate acute from chronic infections and is best used in combination with a battery of other tests. When the AC/HS yields a “nonacute” pattern, the infection has been present for at least 12 months from the time of serum sampling.309

Listeriosis

Serology

The agglutination reaction (Widal’s test) demonstrates antibodies against O and H antigens of the various Listeria serovars. Unfortunately, because of the antigenic complexity of *L. monocytogenes*, no agreement has been reached on the interpretation of agglutination reactions

for diagnostic purposes.

Detection of antibodies to LLO has also been used to diagnose human listeriosis. Purified LLO incorporated into nitrocellulose filters is tested with serial dilutions of sera. Absorbed anti-LLO is identified using enzyme-labeled anti- human IgG. Sensitivity and specificity of the test is over 90%, and during a febrile gastroenteritis outbreak, it correlated well with clinical illness. Although these results are impressive, the technique is not available commercially. A precipitin test,236 indirect hemagglutination reaction, and antigen fixation test238 have also been described, showing apparent success but remaining unavailable on a widespread basis.

Leptospira Species (Leptospirosis) Serology

The macroscopic slide agglutination test is the most useful test for rapid screening.18,19 Twelve serotypes of killed Leptospira (including strains responsible for most infections in the US) are included in this test. The microscopic agglutination test uses live organisms and is the gold standard for detecting antibodies to *Leptospira.*

Generally, agglutination test results are not positive until after the first week of infection; antibody levels peak 3 to 4 weeks after the onset of symptoms and can persist for years, although concentrations may decline over time. Demonstration of at least a fourfold rise in antibodies between acute and convalescent serum samples tested together is most definitive. Newer serologic tests may become useful and include indirect hemagglutination tests, passive microcapsule agglutination test, and enzyme immunoassays.20–24

Blood cell antigens and antibodies: erythrocytes, platelets, and granulocytes6 Tubes

For agglutination tests, use medium-sized (75 × 10 or 12 mm) disposable plastic or glass tubes. Similar tubes should be used for lysis tests when it is essential to have a relatively deep layer of serum to look through, if small amounts of lysis are to be detected. The level of the fluid must rise much higher than the concave bottom of the tubes.

Glass tubes should always be used if the contents are to be heated to 50°C or higher or if organic solvents are being used. Glass tubes, however, are difficult to clean satisfactorily, particularly small-bore tubes, and cleaning methods such as those given on page 695 should be followed carefully.

Toxoplasmosis Agglutination Test

The agglutination test is available commercially in Europe and has been evaluated by a number of investigators. The test employs whole parasites that have been preserved in formalin. The method is very sensitive to IgM antibodies. Nonspecific agglutination (apparently related

to "naturally" occurring IgM *T. gondii* agglutinins) has been observed in persons devoid of antibody in the dye test and conventional IFA test [626]. These natural IgM antibodies do not cross the placenta but are detected at low titers as early as the second month of life. They do not develop in infants with maternal IgG antibody to *T. gondii*, however, so long as IgG antibody is present. False-positive results due to these natural antibodies may be avoided. When they are, this test is excellent for wide-scale screening of pregnant women because it is accurate, simple to perform, and inexpensive. A method that employs latex-tagged particles also may become available commercially.

Introduction to Immunoassay Product Technology in Clinical Diagnostic Testing

Slide agglutination tests are qualitative tests used to detect the presence of antibodies in serology laboratories and blood banks. Treated red blood cells or colored latex beads, coated with antigen, clump in the presence of antibody to the antigen. The degree of clumping may be measured using absorbance at 600 nm, and **latex agglutination** has been applied to quantitative assays.

Precipitin Assays

Precipitin assays, such as radial immunodiffusion and immunoelectrophoresis, are still used for certain applications, but these tend to be low volume assays, in specialist centers. In these types of assays, the presence of antibody (or antigen) in the sample causes the formation of a precipitate in agar containing antigen (or antibody). Radial immunodiffusion involves adding samples to circular holes cut in agar plates and the formation of a circular ring. In immunoelectrophoresis, proteins are first separated by electrophoresis before incubation with antibodies in a parallel trough, with the formation of precipitin arcs.

Complement Fixtion Tests:

The complement fixation test is one of the major traditional tests for the demonstration of presence of specific antigens or antibodies. It requires a veritable zoo of reagents and numerous preparatory steps. There are almost as many versions as there have been users; the microtitre version developed at the Centers for Disease Control and Prevention (LBCF Test) includes rigorous controls and is commonly used.

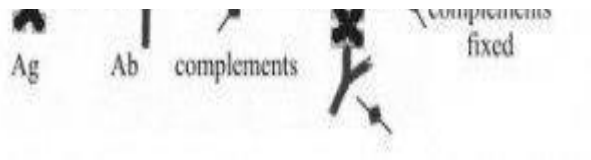

Fig. 4.2 Fixation of Complement

The complement fixation test (CFT) does not depend on hem-agglutinating activity of the virus, but the antibodies must fix the complement, and the sera must be free of anticomplementary activity. The test has been superseded, in many instances, by newer tests, such as enzyme immunoassay (EIA).

The terminal components of the complement cascade, C789 (the membrane attack complex), can damage cell membranes in the presence of specific antibody, which fixes complement to the cell surface. In the CFT, erythrocytes are used as the target cell, because complement-induced leakiness of the membrane can be visualized or measured calorimetrically as an increase in free hemoglobin. In the presence of specific antibodies to an infectious agent, any complement in the system is bound, **leaving no residual complement** for reaction with antibodies to the erythrocytes. Thus, the **presence of specific antibody is indicated by the absence of hemolysis.**

Materials and Reagents

Sheep erythrocytes suspension (5% suspension of washed sheep RBCs) Hemolysin (rabbit anti-sheep red-cell antibody)

Guinea pig complement, free of antibodies to the agent of interest (**Note**:*Guinea pig is the commonest source of fresh complement)*

Barbital-buffered diluents Plastic microtitre plate Centrifuge adapter for microtitre plates Water bath for incubation of plates Color standards for judging hemolysis (prepared by lysing various concentrations of red cells)

Procedure of Complement Fixation Test

Complement Fixation Test (CFT) consists of two stage:

First step (Complement fixation stage): a known antigen and inactivated patient's serum are incubated with a standardized, limited amount of complement.

#Note:patient's serum is heated at 56°C for 30 minutes to inactivate endogenous complement which may disturb the test calibration.

If the **serum contains specific complement activating antibody, the complement will be activated or fixed** by the antigen-antibody complex.

However, if there is **no antibody** in the patient's serum, there will be no formation of antigen-antibody complex, thus complement will not be fixed but **will remain free** (In the indicator stage this complement will react with RBC coated with antibody to sheep RBC).

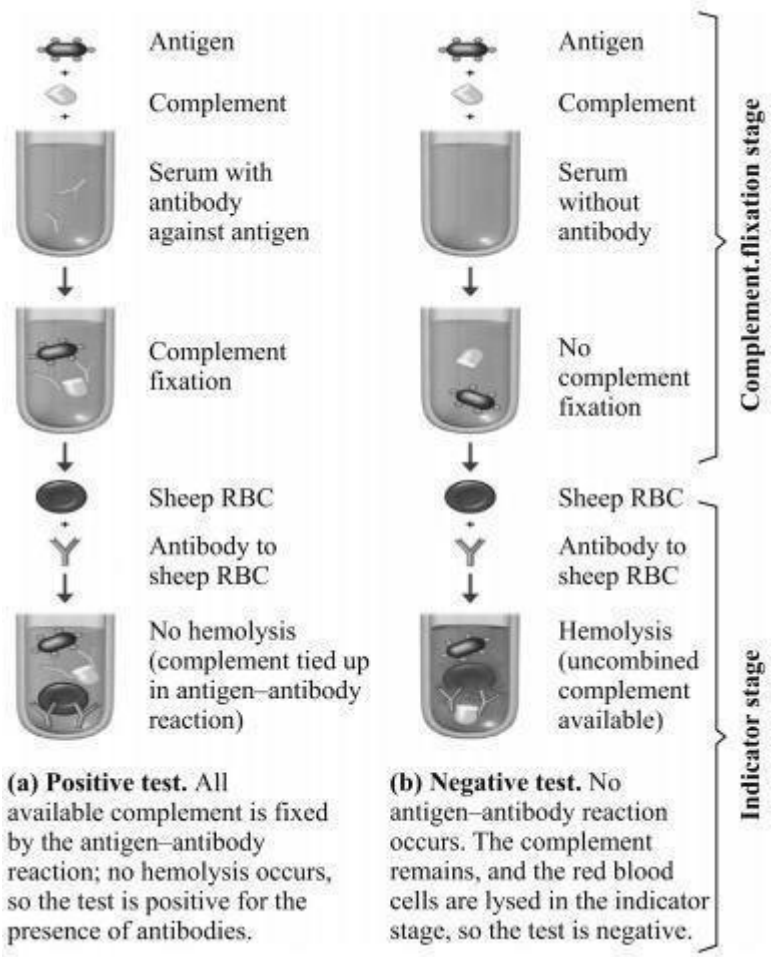

Fig. 4.3 Complement Fixation Test Procedure/Results

Second step (Indicator Stage): The second step detects whether complement has been utilized in the first step or not. This is done by adding the indicator system.

If the complement is fixed in the first step owing to the **presence of antibody** there will be **no complement left to fix to the indicator system**. There won't be any lysis of RBCs.

However, if there is **no** specific antibody in the patient's serum, there will be no antigen-antibody complex, therefore, **complement will be present free or unfixed in the mixture**. This unfixed complement will now react with the antibody- coated sheep RBCs to bring about their lysis.

Results and Interpretation

No lysis of sheep red blood cells (positive CFT) indicates the **presence of antibody** in the test serum, while **lysis** of sheep red blood cells (Negative CFT) indicates the **absence of antibody** in the serum Microtiter Plate, rows 1 and 2 exhibit complement fixation obtained with acute

and convalescent phase serum specimens, respectively. (2-fold serum dilutions were used) The observed 4-fold increase is significant and indicates infection.

Materials required for Quality Control Known positive antibody or antigen Known negative antibody or antigen Serum control without antigen (to detect anticomplementary activity) Antigen controls without serum (to detect anticomplementary activity) Tissue control (the cells or tissue in which the antigen was prepared) Buffer control without antigen or antibody Back titration of complement to document the use of 5CH50 units.

Controls should be used along with the test to ensure that

Antigen and serum are not anti-complimentary
The appropriate amount of complement is used and the sheep red blood cells do not undergo autolysis.

Advantages of Complement Fixation Test

Ability to screen against a large number of viral and bacterial infections at the same time. Economical

Disadvantages of Complement Fixation Test

Not sensitive – cannot be used for immunity screening Time consuming and labor intensive Often non-specific e.g. cross-reactivity between HSV and VZV

Principle of Immunofluorescence

Immunofluorescence is an assay which is used primarily on biological samples and is classically defined as a procedure to detect antigens in cellular contexts using antibodies. The specificity of antibodies to their antigen is the base for immunofluorescence. The biological samples include tissue and cells. Immunofluorescence allows researchers to evaluate whether or not cells in a particular sample express the antigen in question. In cases where an immunopositive signal is found, immunofluorescence also allows researchers to determine which subcellular compartments are expressing the antigen. Immunofluorescence can be used on cultured cell lines, tissue sections, or individual cells. Immunofluorescence may be used to analyze the distribution of proteins, glycans, and small biological and non-biological molecules. Immunofluorescence has been widely used in biological research and medical research yield and becomes one most important and effective method

There are two different immunofluorescence assay which include indirect immunofluorescence assay and direct immunofluorescence assay.For indirect immunofluorescence assay, the protocol mainly include tissue or tell treparation, tissue or cell fixation, serum blocking, primary antibody incubation, marked second antibody incubation, staining, result judgment and

imaging. For direct immunofluorescence assay, there are only marked primary antibody been incubated without second antibody and other steps are same.

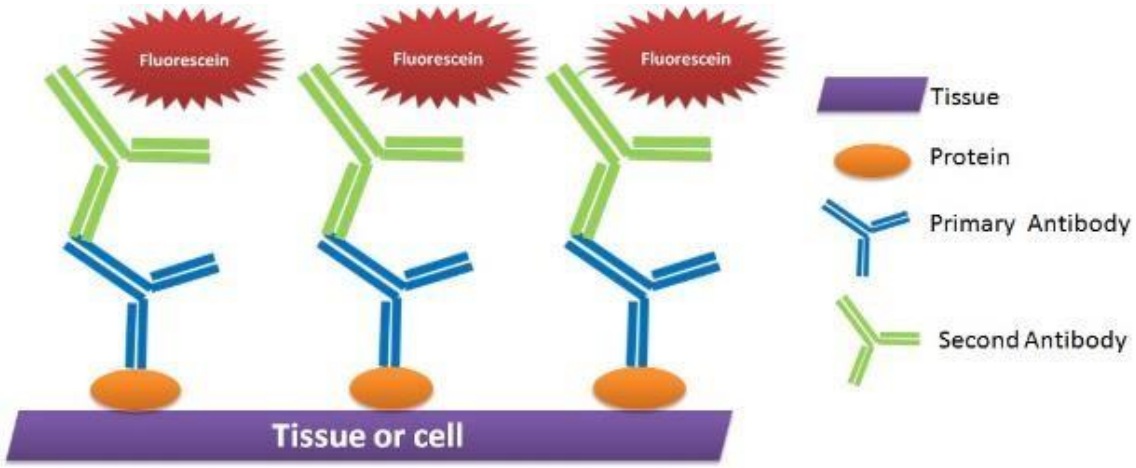

Fig. 4.4 Indirect Immunofluorescence / IF/ ICC principle diagram

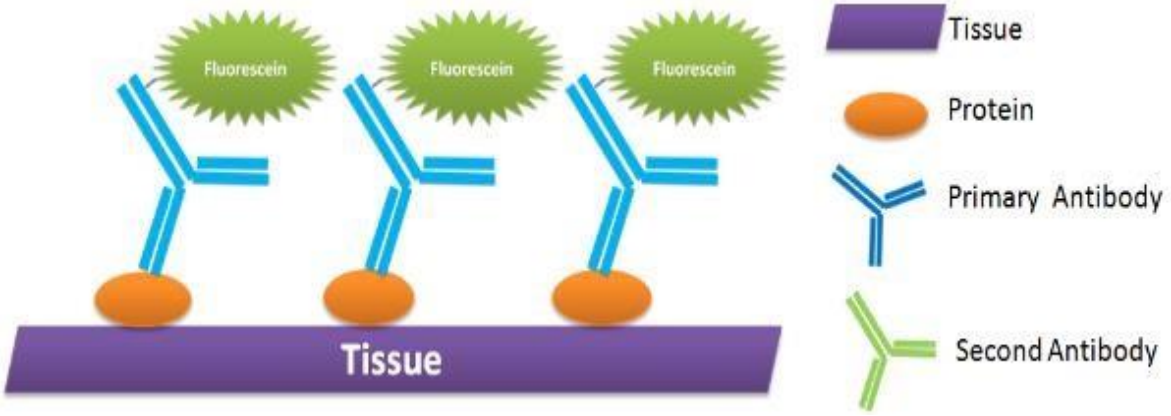

Fig 4.5 Direct Immunofluorescence / IF/ ICC principle diagram

Radioimmunoassay (RIA) method

Radioimmunoassay (RIA) is an in vitro assay that measures the presence of an antigen with very high sensitivity. Basically any biological substance for which a specific antibody exists can be measured, even in minute concentrations.

The target antigen is labeled radioactively and bound to its specific antibodies (a limited and known amount of the specific antibody has to be added). A sample, for example a blood-serum, is then added in order to initiate a competitive reaction of the labeled antigens from the preparation, and the unlabeled antigens from the serum-sample, with the specific antibodies. The competition for the antibodies will release a certain amount of labeled antigen. This amount is proportional to the ratio of labeled to unlabeled antigen. A binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived.

That means that as the concentration of unlabeled antigen is increased, more of it binds to the antibody, displacing the labeled variant. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigens remaining in the supernatant is measured. A binding curve can be generated using a known standard, which allows the amount of antigens in the patient's serum to be derived.

Radioimmunoassay is an old assay technique but it is still a widely used assay and

continues to offer distinct advantages in terms of simplicity and sensitivity.

Needed substances and equipment:

1. Specific antiserum to the antigen to be measured
2. Availability of a radioactive labeled form of the antigen
3. A method in which the antibody-bound tracer can be separated from the unbound tracer
4. An instrument to count radioactivity

Radioactivity:

125-I labels are usually applied although other isotopes such as C14 and H3 have also been used. Usually, high specific activity radio-labeled (125-I) antigen is prepared by iodination of the pure antigen on its tyrosine residue(s) by chloramine-T or peroxidase methods and then separating the radio-labeled antigen from free-isotope by gel-filtration or HPLC. Other important components of RIA are the specific antibody against the antigen and pure antigen for use as the standard or calibrator.

Separation techniques:

Double antibody, charcoal, cellulose, chromatography or solid phase techniques are applied to separate bound and free radio-labeled antigen. Most frequently used is the double antibody technique combined with polyethylene. The bound or free fraction is counted in a gamma counter.

Concomitantly, a calibration or standard curve is generated with samples of known concentrations of the unlabeled standards. The amount of antigen in an unknown sample can be calculated from this curve.

Sensitivity:

The sensitivity can be improved by decreasing the amount of radioactively-labeled antigen and/or antibody. The sensitivity can also be improved by the so-called disequilibrium incubation. In this case radioactively labeled antigen is added after initial incubation of antigen and antibody.

ELISA-ENZYME LINKED IMMUNOSORBENT ASSAY:

What is ELISA (enzyme-linked immunosorbent assay)?

ELISA (enzyme-linked immunosorbent assay) is a plate-based assay technique designed for detecting and quantifying: substances such as peptides, proteins, antibodies and hormones. Other names, such as enzyme immunoassay (EIA), are also used to describe the same

technology. In an ELISA, an antigen must be immobilized on a solid surface and then complexed with an antibody that is linked to an enzyme. Detection is accomplished by assessing the conjugated enzyme activity via incubation with a substrate to produce a measureable product. The most crucial element of the detection strategy is a highly specific antibody-antigen interaction.

ELISAs are typically performed in 96-well (or 384-well) polystyrene plates, which passively bind antibodies and proteins. It is this binding and immobilization of reagents that makes ELISAs so easy to design and perform. Having the reactants of the ELISA immobilized to the microplate surface makes it easy to separate bound from non-bound material during the assay. This ability to wash away nonspecifically bound materials makes the ELISA a powerful tool for measuring specific analytes within a crude preparation.

A detection enzyme or other tag can be linked directly to the primary antibody or introduced through a secondary antibody that recognizes the primary antibody. It can also be linked to a protein such as streptavidin if the primary antibody is biotin labeled. The most commonly used enzyme labels are horseradish peroxidase (HRP) and alkaline phosphatase (AP). Other enzymes have been used as well, but they have not gained widespread acceptance because of limited substrate options. These include β-galactosidase, acetylcholinesterase and catalase. A large selection of substrates is available for performing ELISA with an HRP or AP conjugate. The choice of substrate depends upon the required assay sensitivity and the instrumentation available for signal-detection (spectrophotometer, fluorometer or luminometer).

ELISA formats

ELISAs can be performed with a number of modifications to the basic procedure. The key step, immobilization of the antigen of interest, can be accomplished by direct adsorption to the assay plate or indirectly via a capture antibody that has been attached to the plate. The antigen is then detected either directly (labeled primary antibody) or indirectly (labeled secondary antibody). The most powerful ELISA assay format is the sandwich assay. This type of capture assay is called a “sandwich” assay because the analyte to be measured is bound between two primary antibodies – the capture antibody and the detection antibody. The sandwich format is used because it is sensitive and robust.

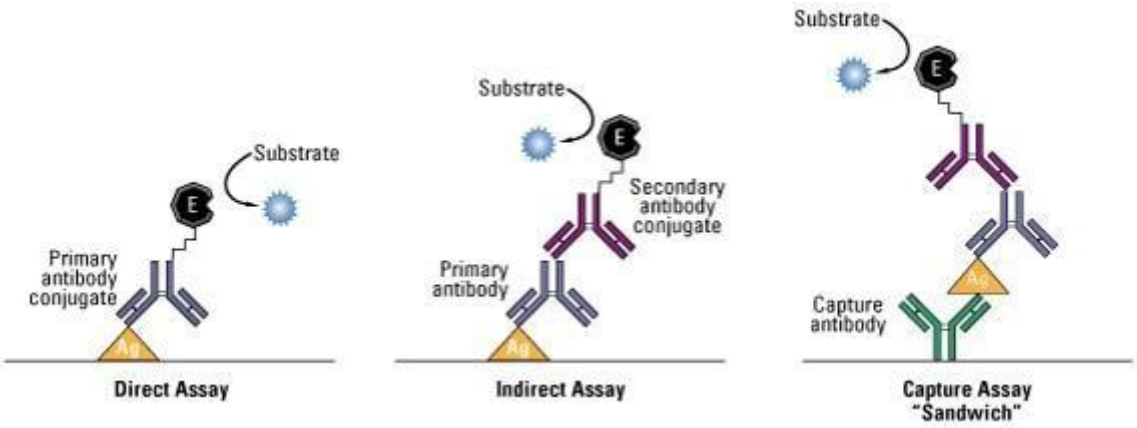

Fig 4.6 Diagram of common ELISA formats (direct vs. sandwich assays).

In the assay, the antigen of interest is immobilized by direct adsorption to the assay plate or by first attaching a capture antibody to the plate surface. Detection of the antigen can then be performed using an enzyme-conjugated primary antibody (direct detection) or a matched set of unlabeled primary and conjugated secondary antibodies (indirect detection).

Direct vs. indirect detection ELISA strategies

Among the standard assay formats discussed and illustrated above, where differences in both *capture* and *detection* were the concern, it is important to differentiate between the particular strategies that exist specifically for the *detection* step. Irrespective of the method by which an antigen is captured on the plate (by direct adsorption to the surface or through a pre-coated "capture" antibody, as in a sandwich ELISA), it is the detection step (as either direct or indirect detection) that largely determines the sensitivity of an ELISA.

The direct detection method uses a labeled primary antibody that reacts directly with the antigen. Direct detection can be performed with an antigen that is directly immobilized on the assay plate or with the capture assay format. Direct detection while not widely used in ELISA is quite common for immunohistochemical staining of tissues and cells.

The indirect detection method uses a labeled secondary antibody for detection and is the most popular format for ELISA. The secondary antibody has specificity for the primary antibody. In a sandwich ELISA, it is critical that the secondary antibody be specific for the detection primary antibody only (and not the capture antibody) or the assay will not be specific for the antigen. Generally, this is achieved by using capture and primary antibodies from different host species (e.g., mouse IgG and rabbit IgG, respectively). For sandwich assays, it is beneficial to use secondary antibodies that have been cross-adsorbed to remove any secondary antibodies that might have affinity for the capture antibody.

Competitive ELISA is a strategy that is commonly used when the antigen is small and has only one epitope, or antibody binding site. One variation of this method consists of labeling purified antigen instead of the antibody. Unlabeled antigen from samples and the labeled antigen compete for binding to the capture antibody. A decrease in signal from the purified antigen indicates the presence of the antigen in samples when compared to assay wells with labeled antigen alone.

ELISPOT (enzyme-linked immunospot assay) refers to ELISA-like capture and measurement of proteins secreted by cells that are plated in PVDF-membrane-backed microplate wells. It is a "sandwich" assay in which the proteins are captured locally as they are secreted by the plated cells, and detection is with a precipitating substrate. ELISPOT is like a western blot in that the result is spots on a membrane surface.

Sandwich ELISA. This experiment was performed using AB finity EGF Recombinant Rabbit Monoclonal Antibody at 2 µg/mL. A standard curve was plotted with full length recombinant EGF protein with concentrations ranging from 0.3 pg/mL to 12.5 ng/mL. An anti-EGF antibody conjugated to biotin was used as a detector at a concentration of 2 µg/mL.

An important consideration in designing a sandwich ELISA is that the capture and detection antibodies must recognize two different non-overlapping epitopes. When the antigen binds to the capture antibody, the epitope recognized by the detection antibody must not be obscured or altered. Capture and detection antibodies that do not interfere with one another and can bind simultaneously are called "matched pairs" and are suitable for developing a sandwich ELISA. Many primary antibody suppliers provide information about epitopes and indicate pairs of antibodies that have been validated in ELISA as matched pairs.

Another design consideration in choosing antibodies is cost. A polyclonal antibody is generally less expensive (~5 fold) to produce than a monoclonal. The specificity gained by using monoclonals for both the capture and detecting antibody must be weighed against the cost and time required for producing two monoclonal antibodies. Preparing a “self- sandwich” ELISA assay, where the same antibody is used for the capture and detection, can limit the dynamic range and sensitivity of the final ELISA.

Cross reaction

Cross-reactivity between antigens occurs when an antibody raised against one specific antigen has a competing high affinity toward a different antigen. This is often the case when two antigens have similar structural regions that the antibody recognizes. Antigens are said to be cross-reactive. Autoantibodies stimulated by external antigens in this way can cause

serious damage. For example, the streptococci that cause rheumatic fever make antigens that are cross-reactive with those on heart muscle membranes, and the antibodies that react with the bacteria also bind to the heart tissue and cause myocardial infraction.

Immunoelectrophoresis

- Immunoelectrophoresis refers to precipitation in agar under an electric field.
- To characterize antibody
- It is a process of a combination of immuno-diffusion and electrophoresis.
- An antigen mixture is first separated into its component parts by electrophoresis and then tested by double immuno-diffusion.
- Antigens are placed into wells cut in a gel (without antibody) and electrophoresed. A trough is then cut in the gel into which antibodies are placed.
- The antibodies diffuse laterally to meet diffusing antigens, and lattice formation and precipitation occur permitting determination of the nature of the antigens.
- The term "immunoelectrophoresis" was first coined by Grabar and Williams in 1953.

Principles

- When an electric current is applied to a slide layered with gel, the antigen mixture placed in wells is separated into individual antigen components according to their charge and size.
- Following electrophoresis, the separated antigens are reacted with specific antisera placed in troughs parallel to the electrophoretic migration and diffusion is allowed to occur.
- Antiserum present in the trough moves toward the antigen components resulting in the formation of separate precipitin lines in 18-24 hrs,
- each indicating reaction between individual proteins with its antibody.

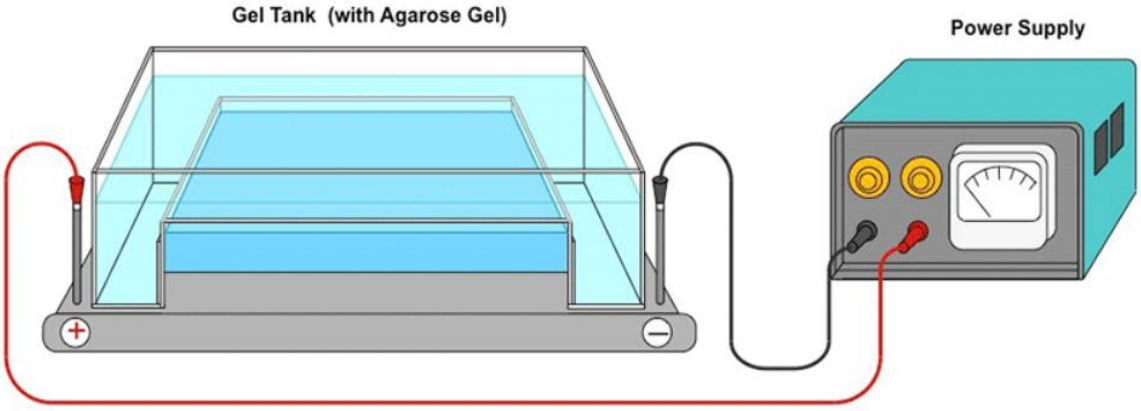

Fig 4.7 Immunoelectrophoretic kit

Procedure

1. Agarose gel is prepared on a glass slide put in a horizontal position.
2. Using the sample template, wells are borne on the application zone carefully.
3. The sample is diluted 2:3 with protein diluent solution (20µl antigen solution +10 µl diluent).
4. Using a 5 µl pipette, 5 µl of control and sample is applied across each corresponding slit (Control slit and Sample slit).
5. The gel is placed into the electrophoresis chamber with the samples on the cathodic side, and electrophoresis runs for 20 mins/ 100 volts.
6. After electrophoresis completes, 20 µl of the corresponding antiserum is added to troughs in a moist chamber and incubated for 18- 20 hours at room temperature in a horizontal position.
7. The agarose gel is placed on a horizontal position and dried with blotter sheets.
8. The gel in saline solution is soaked for 10 minutes and the drying and washing repeated twice again.
9. The gel is dried at a temperature less than 70°C and may be stained with protein staining solution for about 3 minutes followed by decolorizing the gel for 5 minutes in distaining solution baths.
10. The gel is dried and results evaluated.

Results

1. The presence of elliptical precipitin arcs represents antigen-antibody interaction.
2. The absence of the formation of precipitate suggests no reaction.

Different antigens (proteins) can be identified based on the intensity, shape, and position of the precipitation lines.

Application

1. The test helps in the identification and approximate quantification of various proteins present in the serum.
2. Immunoelectrophoresis created a breakthrough in protein identification and in immunology.
3. Immunoelectrophoresis is used in patients with suspected monoclonal and polyclonal gammopathies.
4. The method is used to detect normal as well as abnormal proteins, such as myeloma proteins in human serum.
5. Used to analyze complex protein mixtures containing different antigens.

6. The medical diagnostic use is of value where certain proteins are suspected of being absent (e.g., hypogammaglobulinemia) or overproduced (e.g., multiple myeloma).
7. This method is useful to monitor antigen and antigen-antibody purity and to identify a single antigen in a mixture of antigens.
8. Immunoelectrophoresis is an older method for qualitative analysis of M-proteins in serum and urine.
9. Immunoelectrophoresis aids in the diagnosis and evaluation of the therapeutic response in many disease states affecting the immune system.

Advantages

1. Immunoelectrophoresis is a powerful analytical technique with high resolving power as it combines the separation of antigens by electrophoresis with immunodiffusion against an antiserum.
2. The main advantage of immunoelectrophoresis is that a number of antigens can be identified in serum.

Limitations

1. Immunoelectrophoresis is slower, less sensitive, and more difficult to interpret than Immunofixation electrophoresis.
2. IEP fails to detect some small monoclonal M-proteins because the most rapidly migrating immunoglobulins present in the highest concentrations may obscure the presence of small M-proteins.
3. The use of immunoelectrophoresis in food analysis is limited by the availability of specific antibodies.

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DEPARTMENT OF BIOMEDICAL ENGINEERING

UNIT – V – Introduction to Immunology – SBMA1302

HYPERSENSITIVITY

- Hypersensitivity is increased reactivity or increased sensitivity by the animal body to an antigen to which it has been previously exposed.
- The term is often used as a synonym for allergy, which describes a state of altered reactivity to an antigen.
- Hypersensitivity has been divided into categories based upon whether it can be passively transferred by antibodies or by specifically immune lymphoid cells.
- The most widely adopted current classification is that of Coombs and Gell that designates immunoglobulin-mediated (immediate) hypersensitivity reactions as types I, II, and III, and lymphoid cell-mediated (delayed-type) hypersensitivity/cell-mediated immunity as a type IV reaction.
- "Hypersensitivity" generally represents the "dark side," signifying the undesirable aspects of an immune reaction, whereas the term "immunity" implies a desirable effect.
- A hypersensitive response (HR) is an anti-pathogen response in plants produced by avr-R system activation that leads to alterations in Ca+ flux, MAPK activation, and NO and ROI formation.
- There is rapid necrosis of plant cells in contact with the pathogen.
- This process prevents spread of the pathogen and releases hydrolytic enzymes that facilitate injury to the pathogen's structural integrity.

Causes of Hypersensitivity

Immune responses that are the cause of hypersensitivity diseases may be specific for antigens from different sources:

- Autoimmunity: reactions against self-antigens.
- Reactions against microbes.
- Reactions against non-microbial environmental antigens.

Mechanism of Hypersensitivity

Hypersensitivity diseases are commonly classified according to the type of immune response and the effector mechanism responsible for cell and tissue injury. These mechanisms include some that are predominantly dependent on antibodies and others predominantly dependent on T cells, although a role for both humoral and cell-mediated immunity is often found in many hypersensitivity diseases.

Types of hypersensitivity reactions

The Gell's and Coombs' classification of hypersensitivity reactions considers four types of reactions. Type I, II, and III reactions are basically mediated by antibodies with or without participation of the complement system; type IV reactions are cell-mediated. While in many pathological processes mechanisms classified in more than one of these types of hypersensitivity reactions may be operative, the subdivision of hypersensitivity states into four broad types aids considerably in the understanding of their pathogenesis.

G.H Gell and R.R.A Coomb classified hypersensitivity reactions into four types.

1. Type-I hypersensitivity reaction: IgE antibody mediated
2. Type-II hypersensitivity reaction: Antibodies mediated
3. Type-III hypersensitivity reaction: Antigen-antibody complex mediated
4. Type-IV hypersensitivity reaction: Activated T-cell and cytokines mediated

Type I Immediate reaction

It is caused by IgE antibodies specific for environmental antigens and is the most prevalent type of hypersensitivity disease. Immediate hypersensitivity diseases, commonly grouped under allergy or atopy, are often caused by activation of interleukin-4 (IL-4), IL-5, and IL-13 producing Th2 cells and the production of IgE antibodies, which activate mast cells and eosinophils and induce inflammation.

Some antigens (allergens), such as insect venom, foods, pollen, and dust mite, can induce the formation of IgE antibodies in individuals with a corresponding predisposition. The IgE antibodies bind via Fc receptors to mast cells (sensitization). If the individual is re-exposed to the allergen, cross-linkage of the membrane-bound IgE occurs. This results in the immediate release of mediators (e.g., histamine, kininogen), which induce vasodilation, smooth-muscle contraction, mucus secretion, edema, and/or skin blisters. Most allergens are small proteins that can easily diffuse through the skin or mucosa. They are frequently proteases and are active at very low doses. IL-4 favours differentiation of TH2 cells. The exact mechanism that leads B cells to produce IgE is not known.

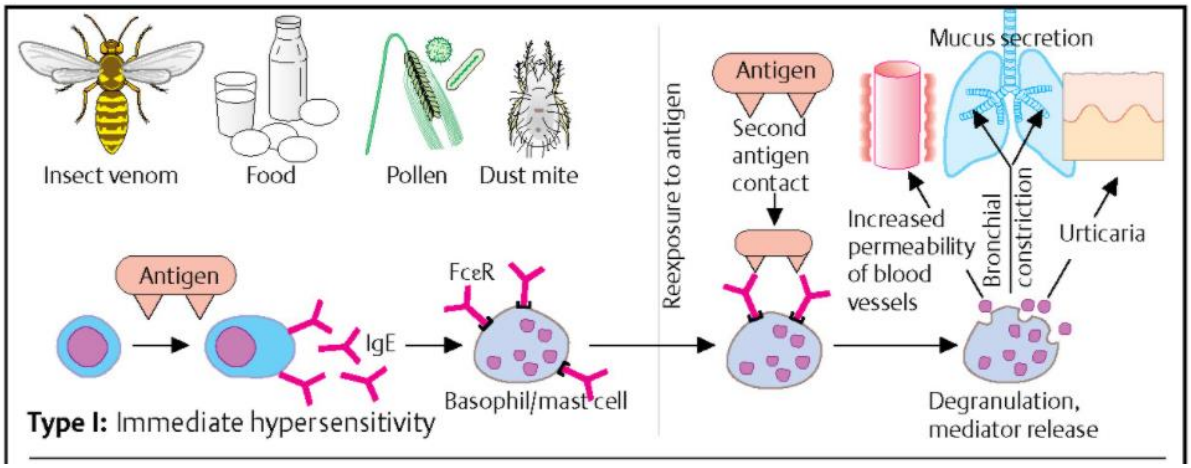


Fig 5.1 Mechanism of Immediate hypersensitivity reaction

Type II: Antibody-mediated cytotoxic reaction

IgG and IgM antibodies specific for cell surface or extracellular matrix antigens can cause tissue injury by activating the complement system, by recruiting inflammatory cells, and by interfering with normal cellular functions. The immunization of individuals to erythrocyte antigens during pregnancy is a typical example of a type II reaction. Children who inherit the RhD erythrocyte antigen from their father can induce immunization against the RhD+ antigen in their RhD-mother. Sensitization usually occurs at birth when fetal blood cells come into contact with the maternal immune system. In any subsequent pregnancies, maternal anti-RhD antibodies of the IgG type can pass into the placenta and cause severe hemolysis of fetal RhD+ erythrocytes.

Other examples: Drugs (e.g., penicillin) can passively bind to erythrocytes. Antibodies directed against penicillin then lead to lysis of the erythrocytes. The formation of antibodies directed against the basement membrane (BM) of the glomerulus can develop during the course of kidney inflammation. Lung damage accompanied by pulmonary hemorrhage and renal inflammation (glomerulonephritis) may occur due to cross-reaction of these antibodies with the basement membrane of the lung (Good-pasture's syndrome).

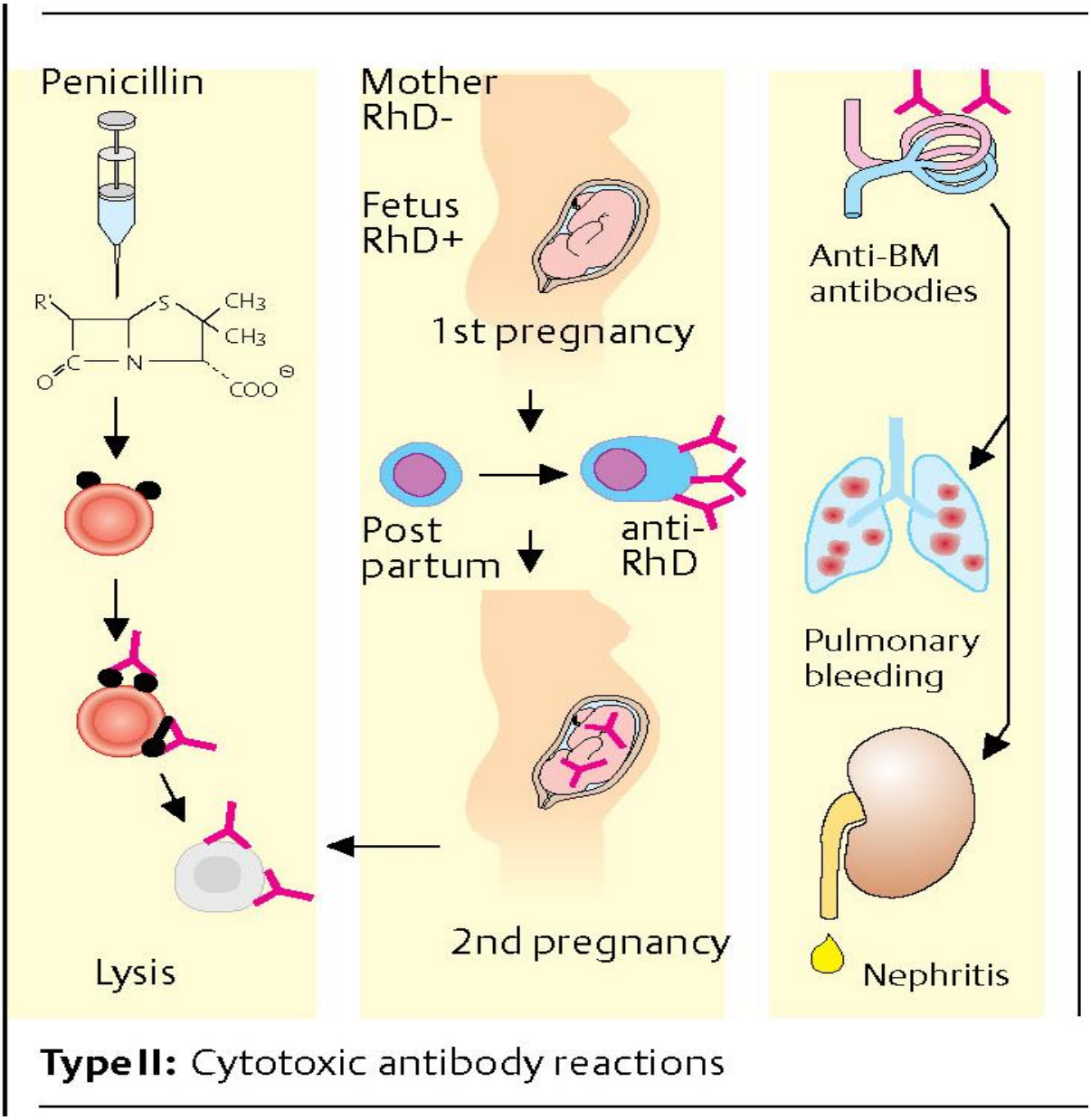


Fig 5. Mechanism of cytotoxic antibody reaction

Type III: Immune complex mediated reactions

IgM and IgG antibodies specific for soluble antigens in the blood form complexes with the antigens, and the immune complexes may deposit in blood vessel walls in various tissues, causing inflammation, thrombosis, and tissue injury. Antibody-antigen complexes (immune complexes) can form during an immune response. Immune complexes can settle in vessel walls, the basement membrane of the lungs and/or kidneys, and in the joints (synovia). They can induce inflammatory processes in these structures by binding complement factors C3a and C5a (anaphylatoxins). A particular type III reaction is the Arthus reaction: when an antigen has penetrated the skin of an individual who has preformed IgG antibodies, the immune complexes can bind to Fc receptors of most cells inducing degranulation inflammatory cells are recruited

and complement is activated, leading to the release of C5a and local inflammation, platelet accumulation, and eventually to blood vessel occlusion with necrosis.

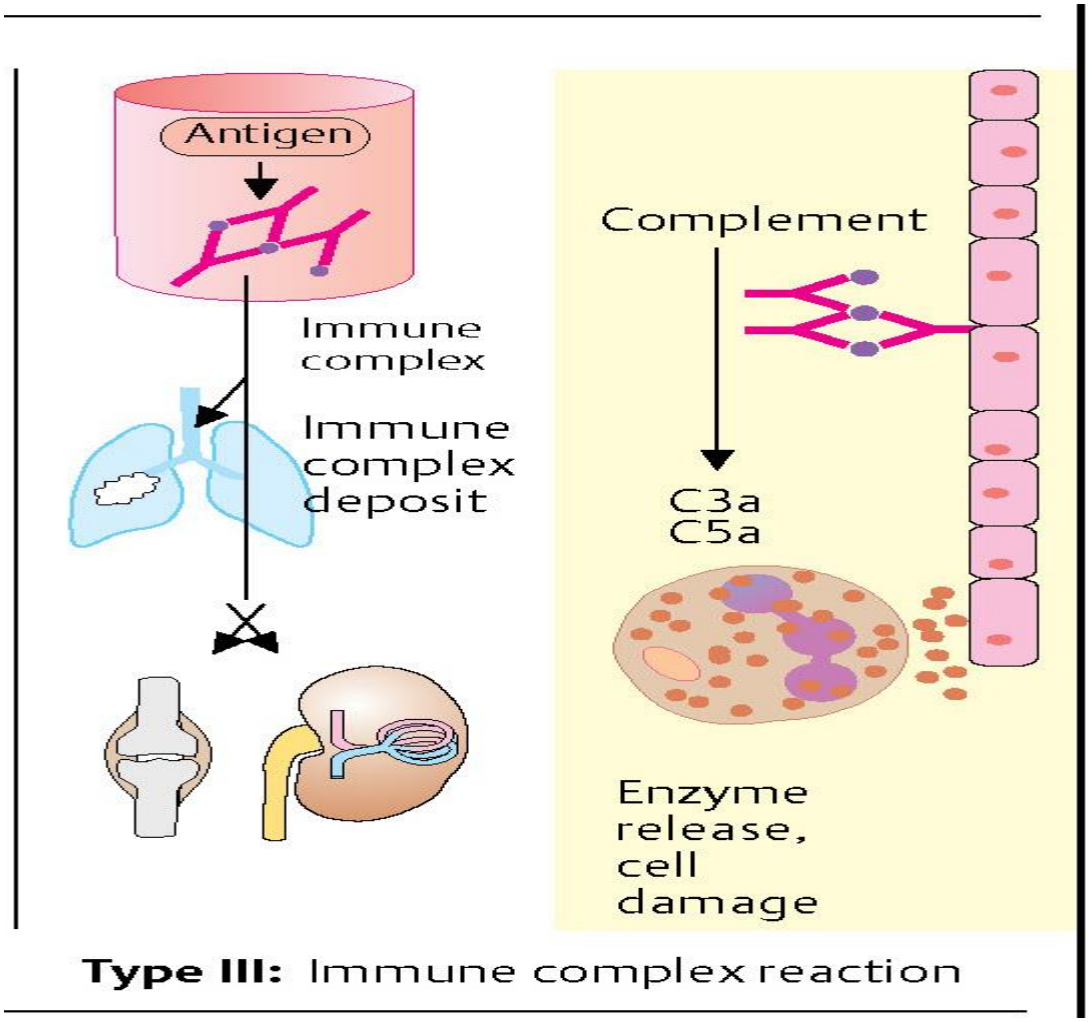


Fig 5. Mechanism of Immune complex reaction

Type IV: Delayed-type hypersensitivity reactions

In these disorders, tissue injury may be due to T lymphocytes that induce inflammation or directly kill target cells. In most of these diseases, the major mechanism involves the activation of CD4+ helper T cells, which secrete cytokines that promote inflammation and activate leukocytes, mainly neutrophils and macrophages. CTLs contribute to tissue injury in some diseases. Haptens are molecules of very small molecular weight (often < 1 kDa). They are too small to function as antigens, but they can penetrate the epidermis and bind to certain proteins in the skin (carrier proteins). Hapten-carrier complexes are bound by antigen-presenting cells of the skin (Langerhans cells), which then migrate to regional lymph nodes. T-cell stimulation then occurs at the lymph node. The so-called sensitization phase lasts ca. 10-14 days. If the individual is reexposed to the hapten, antigen-specific T cells migrate to the skin, where they

accumulate and proliferate. They also cause edema formation and local inflammation with the help of cytokines. Compounds containing nickel or chrome and chemicals such as those found in rubber are typical triggers of type IV hypersensitivity reactions.

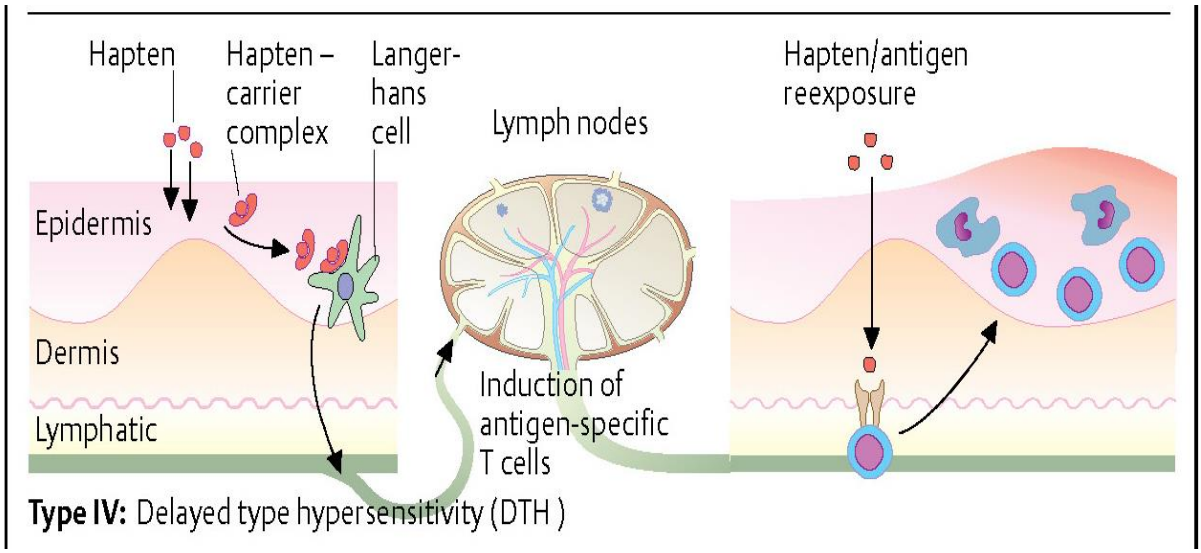


Fig 5.4 Mechanism of delayed type hypersensitivity reaction

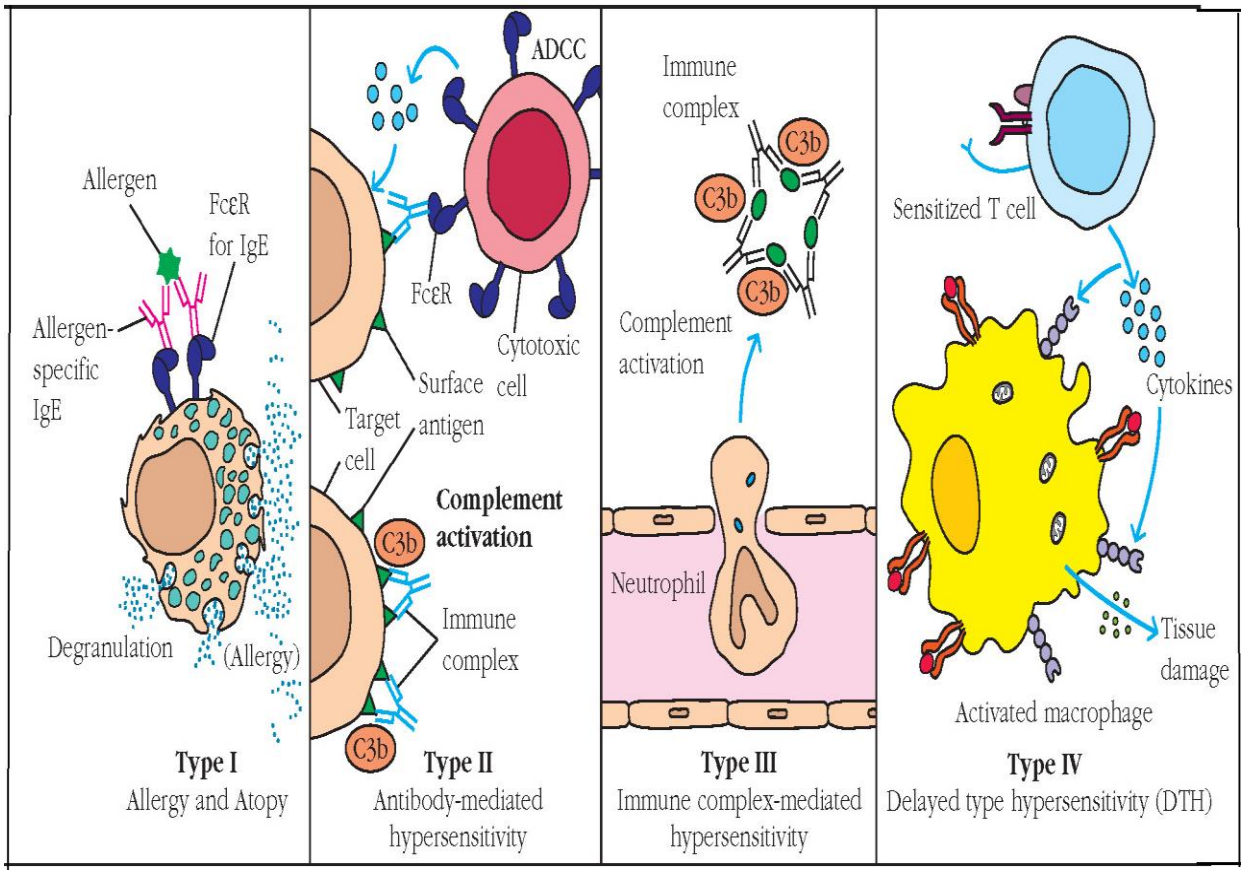


Fig 5.5 Mechanism of hypersensitivity reaction

Transplantation Immunology

Introduction

Today, kidney, pancreas, heart, lung, liver, bone marrow, and cornea transplantations are performed among non-identical individuals with ever increasing frequency and success

Classification

Autologous grafts (Autografts)

- Grafts transplanted from one part of the body to another in the same individual

• **Syngeneic grafts (Isografts)**

- Grafts transplanted between two genetically identical individuals of the same species

Allogeneic grafts (Allografts)

- Grafts transplanted between two genetically different individuals of the same species

Xenogeneic grafts (Xenografts)

- Grafts transplanted between individuals

of different species Graft Rejection

• Grafts rejection is a kind of specific immune response

- Specificity
- Immune memory

• Grafts rejection

- First set rejection
- Second set rejection

Immunologic Basis of Allograft Rejection

I. Transplantation antigens

- Major histocompatibility antigens (MHC molecules)
- Minor histocompatibility antigens
- Other alloantigens

1. Major histocompatibility antigens

a. Main antigens of grafts rejection

b. Cause fast and strong rejection

c. Difference of HLA types is the main cause of human grafts rejection

2. Minor histocompatibility antigens

II. Mechanism of allograft rejection

Cell-mediated Immunity

Recipient's T cell-mediated cellular immune response against alloantigens on grafts

Molecular Mechanisms of Allogeneic Recognition:

- T cells of the recipient recognize the allogenetic MHC molecules
- Many T cells can recognize allogenetic MHC molecules
- 10^{-5}-10^{-4} of specific T cells recognize conventional antigens
- 1%-10% of T cells recognize allogenetic MHC molecules
- The recipient' T cells recognize the allogenetic MHC molecules

Direct Recognition

- Recognition of an intact allogenetic MHC molecule displayed by donor APC in the graft
- Cross recognition
- An allogenetic MHC molecule with a bound peptide can mimic the determinant formed by a self MHC molecule plus foreign peptide
- A cross-reaction of a normal TCR, which was selected to recognize a self MHC molecules plus foreign peptide, with an allogenetic MHC molecule plus peptide

Passenger leukocytes

- Donor APCs that exist in grafts, such as DC, MΦ
- Early phase of acute rejection？
- Fast and strong？
- Many T cells can recognize allogenetic MHC molecules
- Allogenetic MHC molecules (different residues)
- Allogenetic MHC molecules–different peptides
- All allogenetic MHC molecules on donor APC can be epitopes recognized by TCR

Indirect Recognition

- Uptake and presentation of allogeneic donor MHC molecules by recipient APC in "normal way"
- Recognition by T cells like conventional foreign antigens

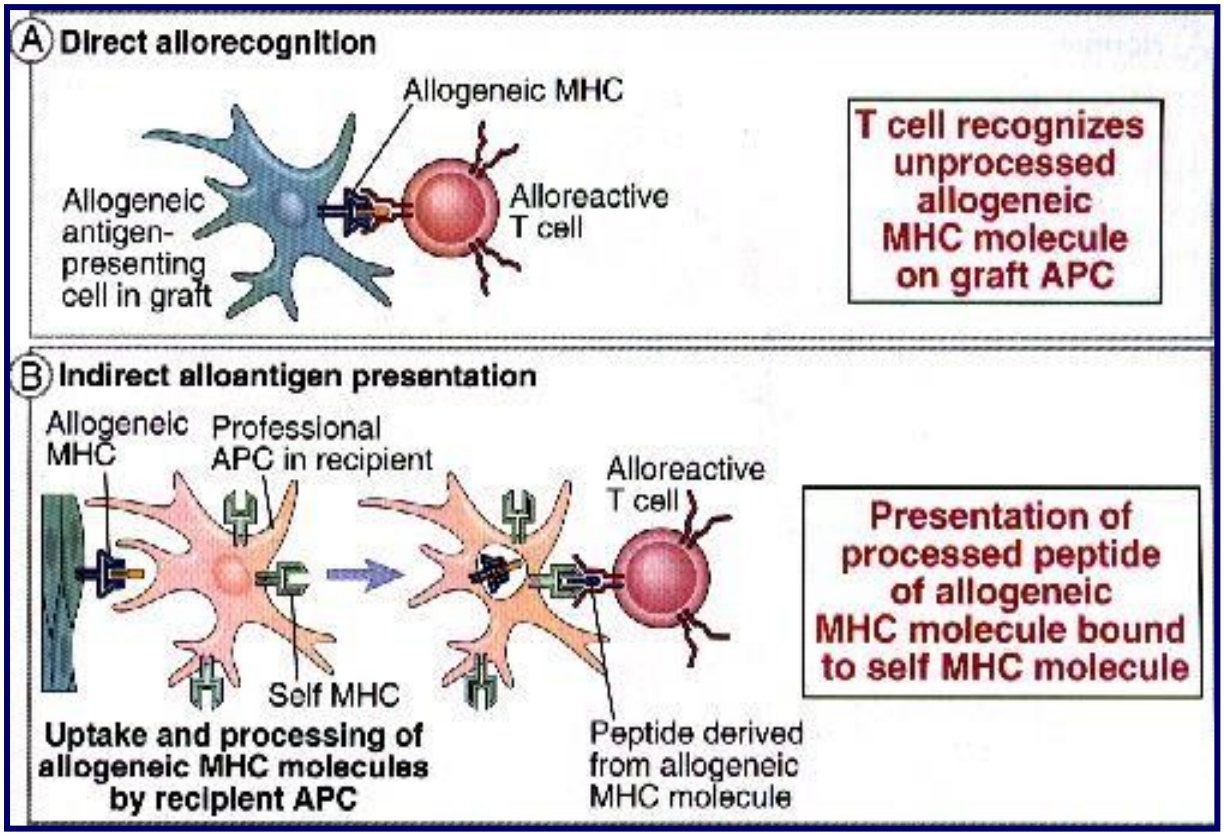


Fig. 5.6 Recognition of graft alloantigen

Classification and Effector Mechanisms of Allograft Rejection Classification of Allograft Rejection

- Host versus graft reaction (HVGR)
- Conventional organ transplantation
- Graft versus host reaction (GVHR)
- Bone marrow transplantation
- Immune cells transplantation
- **Host versus graft reaction (HVGR)**

Hyperacute rejection

- Occurrence time
- Occurs within minutes to hours after host blood vessels are anastomosed to graft vessels
- Pathology
- thrombotic occlusion of the graft vasculature
- Ischemia, denaturation, necrosis

Mechanism

- Preformed antibodies
- Antibody against ABO blood type antigen
- Antibody against VEC (Vascular Endothelial Cell) antigen
- Antibody against HLA antigen
- Complement activation
- Endothelial cell damage
- Platelets activation
- Thrombosis, vascular occlusion, ischemic damage

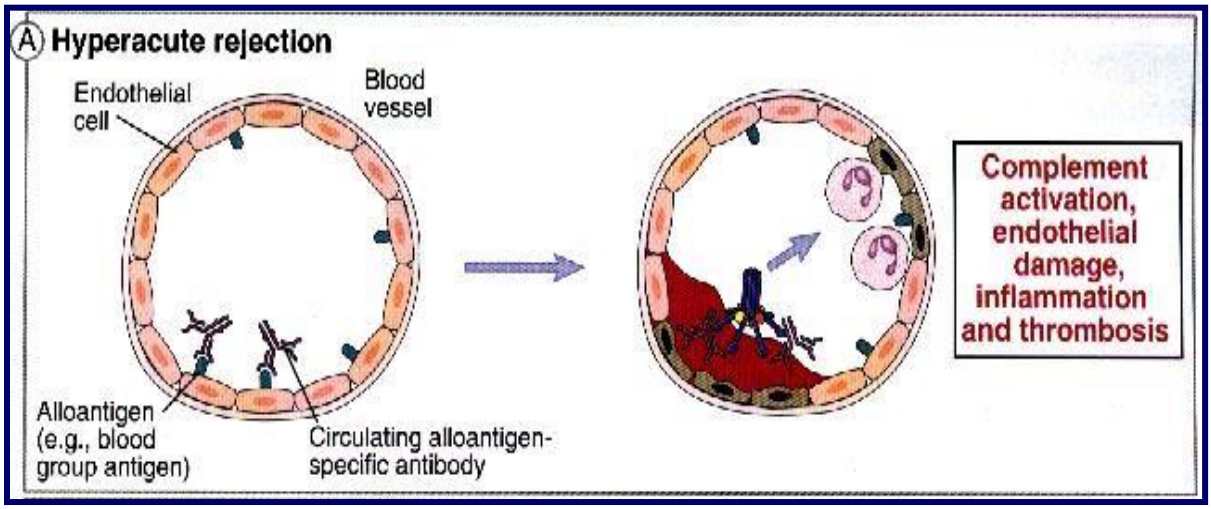


Fig 5.7 Mechanism of hyperacute rejection

Acute rejection

- Occurrence time
- Occurs within days to 2 weeks after transplantation, 80-90% of cases occur within 1 month

Pathology

- Acute humoral rejection
- Acute vasculitis manifested mainly by endothelial cell damage
- Acute cellular rejection
- Parenchymal cell necrosis along with infiltration of lymphocytes and MΦ

Mechanisms

- Vasculitis
- IgG antibodies against alloantigens on endothelial cell

- CDC
- Parenchymal cell damage
- Delayed hypersensitivity mediated by CD4+Th1
- Killing of graft cells by CD8+Tc

Chronic rejection

- Occurrence time
- Develops months or years after acute rejection reactions have subsided

Pathology

- Fibrosis and vascular abnormalities with loss of graft function

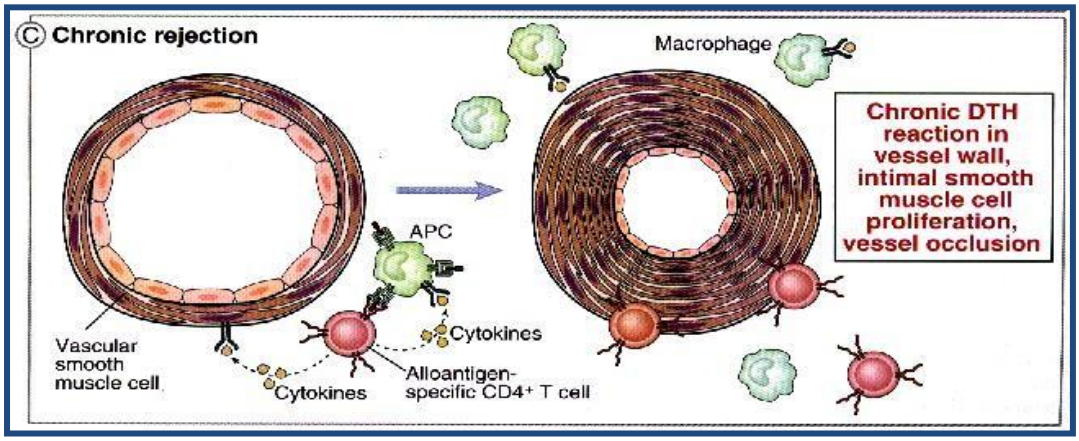


Fig 5.8 Mechanism of chronic rejection

Mechanisms

- Not clear
- Extension and results of cell necrosis in acute rejection
- Chronic inflammation mediated by CD4+T cell/MΦ
- Organ degeneration induced by non immune factors

Graft versus host reaction (GVHR)

- Allogenetic bone marrow transplantation
- Rejection to host alloantigens
- Mediated by immune competent cells in bone marrow

Graft versus host disease (GVHD)

- A disease caused by GVHR, which can damage the host

Acute GVHD

- Endothelial cell death in the skin, liver, and gastrointestinal tract

- Rash, jaundice, diarrhea, gastrointestinal hemorrhage
- Mediated by mature T cells in the grafts

Chronic GVHD

- Fibrosis and atrophy of one or more of the organs
- Eventually complete dysfunction of the affected organ
- Both acute and chronic GVHD are commonly treated with intense immunosuppresion

Prevention and Therapy of Allograft Rejection

- **Tissue Typing**
- ABO and Rh blood typing
- Crossmatching (Preformed antibodies)
- HLA typing
  - HLA-A and HLA-B
  - HLA-DR

•Immunosuppressive Therapy

- Cyclosporine(CsA), FK506
  - Inhibit NFAT transcription factor
- Azathioprine, Cyclophosphamide
  - Block the proliferation of lymphocytes
- Ab against T cell surface molecules
  - Anti-CD3 mAb ---Deplete T cells
- Anti-inflammatory agents
  - Corticosteroids--- Block the synthesis and secretion of cytokines

Immunosuppression involves an act that reduces the activation or efficacy of the immune system. Some portions of the immune system itself have immuno-suppressive effects on other parts of the immune system, and immunosuppression may occur as an adverse reaction to treatment of other conditions.

In general, deliberately induced immunosuppression is performed to prevent the body from rejecting an organ transplant, treating graft-versus-host disease after a bone marrow transplant, or for the treatment of auto-immune diseases such as rheumatoid arthritis or Crohn's disease. This is typically done using drugs, but may involve surgery (splenectomy), plasmapharesis, or radiation.A person who is undergoing immunosuppression, or whose immune system is weak for other reasons (for example, chemotherapy and HIV), is said to be *immunocompromised*.

An **immunosuppressant** is any agent that causes immunosuppression, including immunosuppressive drugs and some environmental toxins.

Immunosuppressive drug

Immunosuppressive drugs or **immunosuppressive agents** or **antirejection medications** are drugs that inhibit or prevent activity of the immune system. They are used in immunosuppressive therapy to:

Prevent the rejection of transplanted organs and tissues (e.g., bone marrow, heart, kidney, liver. Treat autoimmune diseases or diseases that are most likely of autoimmune origin (e.g., rheumatoid arthritis, multiple sclerosis, myasthenia gravis, systemic lupus erythematosus, sarcoidosis, focal segmental glomerulosclerosis, Crohn's disease, Behcet's Disease, pemphigus, and ulcerative colitis). Treat some other non-autoimmune inflammatory diseases (e.g., long term allergic asthma control).

common side-effect of many immunosuppressive drugs is immunodeficiency, because the majority of them act non-selectively, resulting in increased susceptibility to infections and decreased cancer immunosurveillance. There are also other side-effects, such as hypertension, dyslipidemia, hyperglycemia, peptic ulcers, lipodystrophy, moon face, liver and kidney injury. The immunosuppressive drugs also interact with other medicines and affect their metabolism and action. Actual or suspected immunosuppressive agents can be evaluated in terms of their effects on lymphocyte subpopulations in tissues using immunohistochemistry.

Immunosuppressive drugs can be classified into five groups:

- glucocorticoids
- cytostatics
- antibodies
- drugs acting on immunophilins
- other drugs.

1. Glucocorticoids:

In pharmacologic (supraphysiologic) doses, glucocorticoids are used to suppress various allergic, inflammatory, and autoimmune disorders. They are also administered as posttransplantory immunosuppressants to prevent the acute transplant rejection and graft-versus-host disease. Nevertheless, they do not prevent an infection and also inhibit later reparative processes.

1.1. Immunosuppressive mechanism

Glucocorticoids suppress the cell-mediated immunity. They act by inhibiting genes that code

for the cytokines Interleukin 1 (IL-1), IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, and TNF-γ, the most important of which is IL-2. Smaller cytokine production reduces the T cell proliferation.

Glucocorticoids also suppress the humoral immunity, causing B cells to express smaller amounts of IL-2 and IL-2 receptors. This diminishes both B cell clone expansion and antibody synthesis.

1.2. Anti-inflammatory effects

Glucocorticoids influence all types of inflammatory events, no matter their cause. They induce the lipocortin-1 (annexin-1) synthesis, which then binds to cell membranes preventing the phospholipase A2 from coming into contact with its substrate arachidonic acid. This leads to diminished eicosanoid production. The cyclooxygenase (both COX-1 and COX-2) expression is also suppressed, potentiating the effect.

Glucocorticoids also stimulate the lipocortin-1 escaping to the extracellular space, where it binds to the leukocyte membrane receptors and inhibits various inflammatory events: epithelial adhesion, emigration, chemotaxis, phagocytosis, respiratory burst, and the release of various inflammatory mediators (lysosomal enzymes, cytokines, tissue plasminogen activator, chemokines, etc.) from neutrophils, macrophages, and mastocytes.

2. Cytostatics:

Cytostatics inhibit cell division. In immunotherapy, they are used in smaller doses than in the treatment of malignant diseases. They affect the proliferation of both T cells and B cells. Due to their highest effectiveness, purine analogs are most frequently administered.

2.1. Alkylating agents

The alkylating agents used in immunotherapy are nitrogen mustards (cyclophosphamide), nitrosoureas, platinum compounds, and others. Cyclophosphamide (Baxter's Cytoxan) is probably the most potent immunosuppressive compound. In small doses, it is very efficient in the therapy of systemic lupus erythematosus, autoimmune hemolytic anemias, Wegener's granulomatosis, and other immune diseases. High doses cause pancytopenia and hemorrhagic cystitis.

2.2. Antimetabolites

Antimetabolites interfere with the synthesis of nucleic acids. These include:

- folic acid analogues, such as methotrexate
- purine analogues, such as azathioprine and mercaptopurine
- pyrimidine analogues, such as fluorouracil
- protein synthesis inhibitors.

Methotrexate

Methotrexate is a folic acid analogue. It binds dihydrofolate reductase and preventssynthesis of tetrahydrofolate. It is used in the treatment of autoimmune diseases (for example rheumatoid arthritis or Behcet's Disease) and in transplantations.

Azathioprine and mercaptopurine

Azathioprine (Prometheus' Imuran), is the main immunosuppressive cytotoxic substance. It is extensively used to control transplant rejection reactions. It is nonenzymatically cleaved to mercaptopurine, that acts as a purine analogue and an inhibitor of DNA synthesis. Mercaptopurine itself can also be administered directly.

By preventing the clonal expansion of lymphocytes in the induction phase of the immune response, it affects both the cell and the humoral immunity. It is also efficient in the treatment of autoimmune diseases.

Cytotoxic antibiotics

Among these, dactinomycin is the most important. It is used in kidney transplantations. Other cytotoxic antibiotics are anthracyclines, mitomycin C, bleomycin, mithramycin.

TUMOUR IMMUNOLOGY

Cancer immunology is an interdisciplinary branch of biology that is concerned with understanding the role of the immune system in the progression and development of cancer; the most well-known application is cancer immunotherapy, which utilizes the immune system as a treatment for cancer. Cancer immunosurveillance and immunoediting are based on protection against development of tumors in animal systems and (ii) identification of targets for immune recognition of human cancer.

Cancer immunology is an interdisciplinary branch of biology concerned with the role of the immune system in the progression and development of cancer; the most well-known application is cancer immunotherapy, where the immune system is used to treat cancer.Cancer immunosurveillance is a theory formulated in 1957 by Burnet and Thomas, who proposed that lymphocytes act as sentinels in recognizing and eliminating continuously arising, nascent transformed cells. Cancer immunosurveillance appears to be an important host protection process that decreases cancer rates through inhibition of carcinogenesis and maintaining of regular cellular homeostasis. It has also been suggested that immunosurveillance primarily functions as a component of a more general process of cancer immunoediting.

Tumor antigens

Tumors may express tumor antigens that are recognized by the immune system and may induce an immune response. These tumor antigens are either TSA (Tumor-specific antigen) or TAA

(Tumor-associated antigen)

Tumor-specific antigens (TSA)

Tumor-specific antigens are antigens that only occur in tumor cells.TSAs can be products of oncoviruses like E6 and E7 proteins of Human papillomavirus, occurring in cervical carcinoma, or EBNA-1 protein of EBV, occurring in Burkitt's lymphoma cells. Another example of TSAs are abnormal products of mutated oncogenes (e.g. Ras protein) and anti- oncogenes

Tumor-associated antigens (TAA)

Tumor-associated antigens are present in healthy cells, but for some reason they also occur in tumor cells However, they differ in quantity, place or time period of expression. Oncofetal antigens are tumor-associated antigens expressed by embryonic cells and by tumors.Examples of oncofetal antigens are AFP (α-fetoprotein), produced by hepatocellular carcinoma, or CEA (carcinoembryonic antigen), occurring in ovarian and colon cancer. More tumor-associated antigens are HER2/neu, EGFR or MAGE-1.

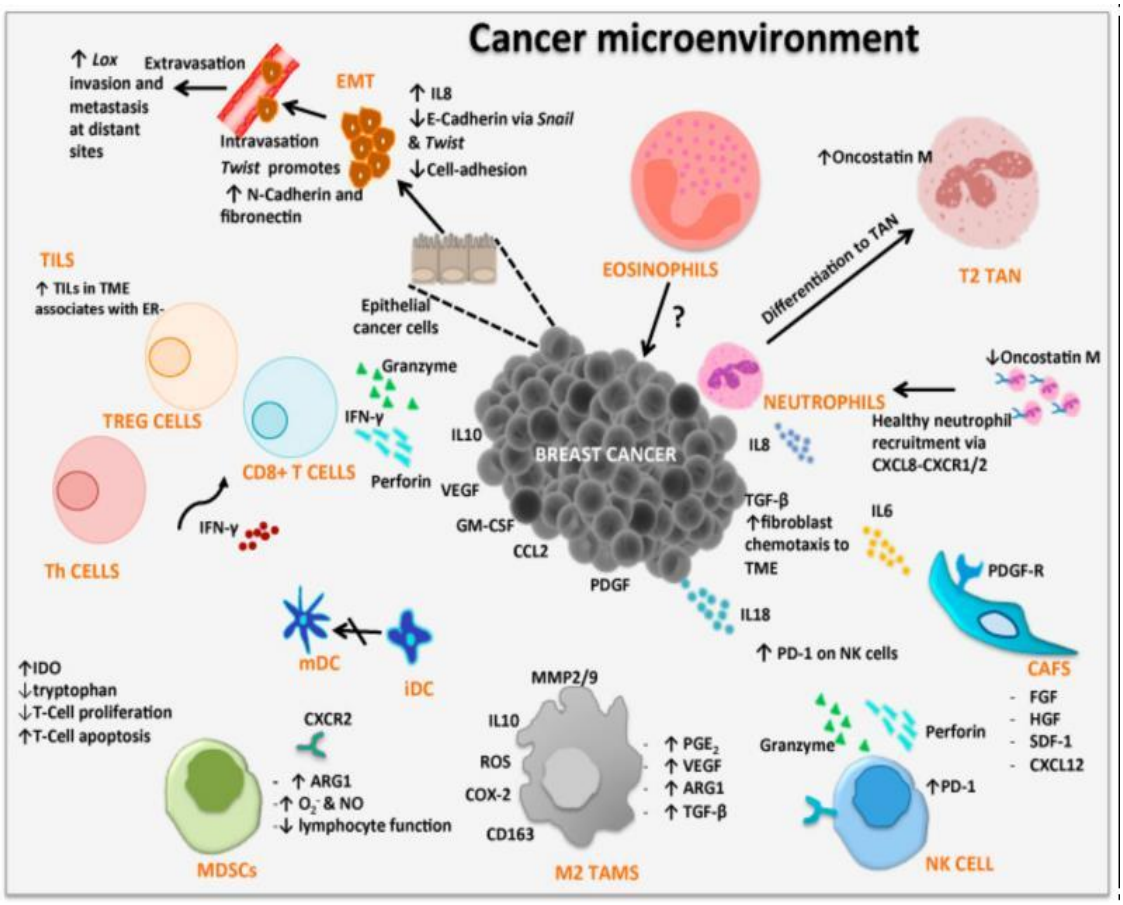


Fig 5.9 Cancer microenvironment

CD8+ cytotoxic T cells are a fundamental element of anti-tumor immunity. Their TCR receptors recognise antigens presented by MHC class I and when bound, the Tc cell triggers its cytotoxic activity. MHC I is present on the surface of all nucleated cells. However, some cancer

cells lower their MHC I expression and avoid being detected by the cytotoxic T cells.This can be done by mutation of MHC I gene or by lowering the sensitivity to IFN-γ (which influences the surface expression of MHC I). Tumor cells also have defects in antigen presentation pathway, what leads into down-regulation of tumor antigen presentations. Defects are for example in Transporter associated with antigen processing (TAP) or Tapasin On the other hand, a complete loss of MHC I is a trigger for NK cells. Tumor cells therefore maintain a low expression of MHC I.

- Another way to escape cytotoxic T cells is to stop expressing molecules essential for co-stimulation of cytotoxic T cells, such as CD80 or CD86.
- Tumor cells express molecules to induce apoptosis or to inhibit T lymphocytes:
  - Expression of FasL on its surface, tumor cells may induce apoptosis of T lymphocytes by FasL-Fas interaction.
  - Expression of PD-L1 on the surface of tumor cells leads to suppresion of T lymphocytes by PD1-PD-L1 interaction.
- Tumor cells have gained resistance to effector mechanisms of NK and Cytotoxic CD8+ T cell:
  - by loss of gene expression or inhibiton of apoptotic signal pathway molecules: APAF1, Caspase 8, Bcl-2-associated X protein (bax) and Bcl-2 homologous antagonist killer (bak).[*citation needed*]
  - by induction of expression or overexpression of antiapoptotic molecules: Bcl- 2, IAP or XIAP.
- Tumor cells produce special cytokines (such as Colony-stimulating factor) to produce Myeloid-derived suppressor cell. These cells are heterogenous collection of cell types including precursors of Dendritic cell, Monocyte and Neutrophil. MDSC have suppressive effects on T-lymphocytes, Dendritic cells and Macrophages. They produce immunosupressive TGF-β and IL-10.
- Another producer of suppresive TGF-β and IL-10 are Tumor-associated macrophages, these macrophages have mostly phenotype of alternatively activated M2 macrophages. Their activation is promoted by TH type 2 cytokines (such as IL-4 and IL-13). Their main effects are immunosuppresion, promotion of tumor growth and angiogenesis.
- Tumor cells have non-classical MHC class I on their surface, for example HLA-G. HLA-G is inducer of Treg, MDSC, polarise macrophages into alternatively

activated M2 and has other immunosuppresive effects on immune cells.

THE ASSOCIATION OF AUTOIMMUNITY WITH DISEASE

The immune system has tremendous diversity and because the repertoire of specificities expresses by the B- and T-cell populations is generated randomly, it is bound to include many which are specific for self-components. Thus the body must establish self-tolerance mechanisms, to distinguish between self and non-self-determinants, so as to avoid autoreactivity (see Chapter 7). However, al mechanism has a risk of breakdown. The selfrecognition mechanisms are no exception, and a number of diseases have been identified in which there is autoimmunity, due to copious production of autoantibodies and autoreactive T cells. One of the earliest examples in which the production of autoantibodies was associated with disease in a given organ is Hashimoto's thyroiditis. Among the autoimmune diseases, thyroiditis has been particularly well-studied, and many of the aspects discussed in this chapter will draw upon our knowledge of it. It is a disease of the thyroid which is most common in middle-aged women and often lead to formation of a goiter and hypothyroidism. The gland is infiltrated, sometimes to an extraordinary extent, with inflammatory lymphoid cells. These are predominantly mononuclear phagocytes, lymphocytes and plasma cells, and secondary lymphoid follicles are common (Figure-1). In Hashimoto's disease, the gland often shows regenerating thyroid follicles but this is not a feature of the thyroid in the related condition, primary myxoedema, in which comparable immunology features are seen and where the gland undergoes almost complete destruction and shrinks. The serum of patients with Hashimoto's disease usually contains antibodies to thyroglobulin. These antibodies are demonstrable by agglutination and by precipitin reactions when present in high titre. Most patients also have anti bodies directed against a cytoplasmic or microsome antigen, also present on the apical surface of the follicular epithelial cells (Figure-2), and now known to be thyroid peroxidase, the enzyme which iodinates thyroglobulin. Although the non-organ-specific disease characteristically produce symptoms in the skin, joints, kidney and muscle, individual organs are more markedly affected by particular diseases, for example the kidney in systemic lupus erythematosus (SLE) and the joints in rheumatoid arthritis.

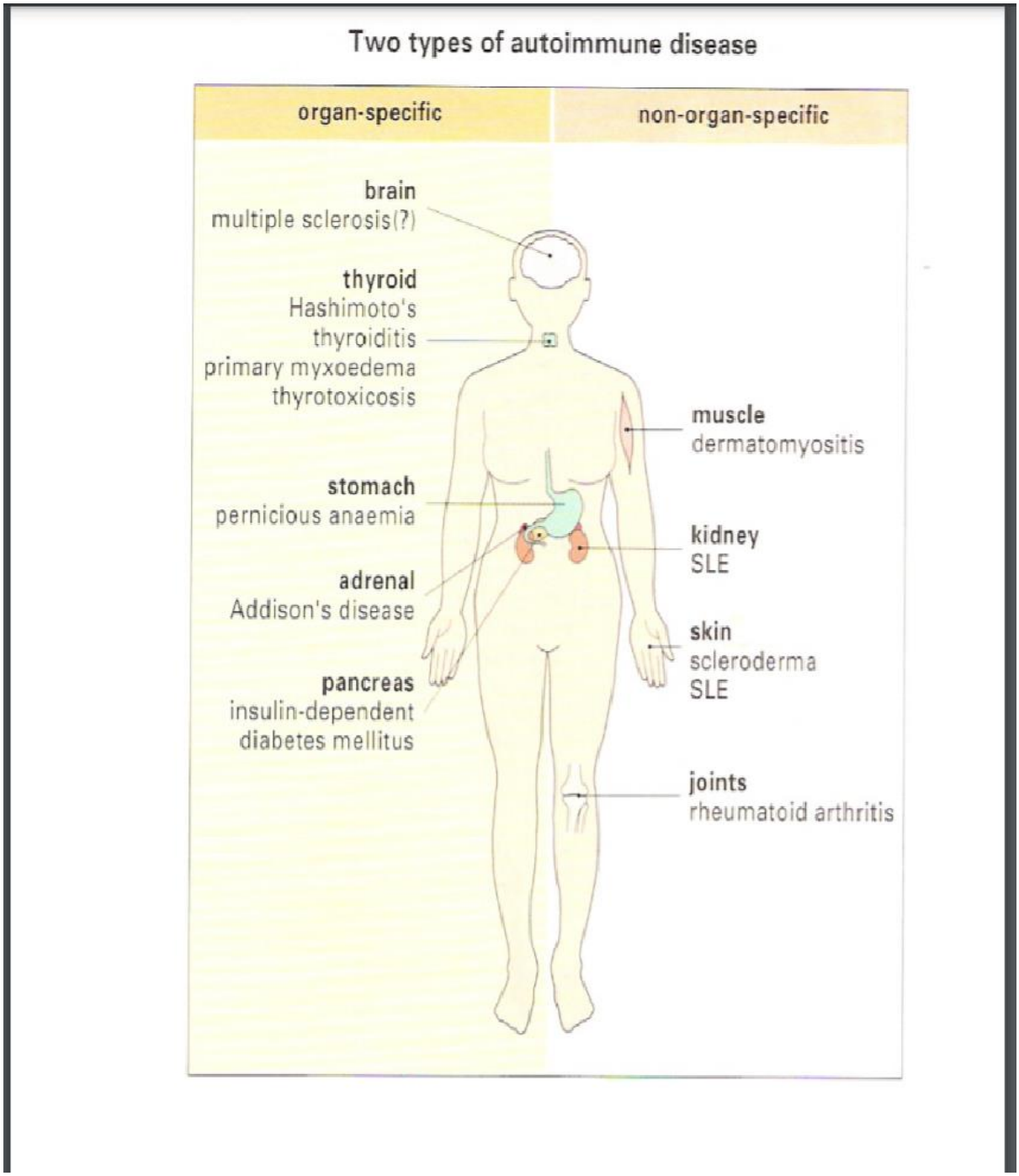


Fig. 5.10 Systemic and organ specific autoimmune disease

Autoimmunity and autoimmune disease

Autoimmune mechanisms underline many diseases, some organ-specific, others systemic in distribution. *ƒ* Autoimmune disorders can overlap: an individual may have more than one organspecific disorder; or more than one systemic disease. *ƒ* Genetic factors such as HLA type are important in autoimmune disease, and it is probable that each disease involves several factors. *ƒ* Autoimmune mechanisms are pathogenic in experimental and spontaneous animal models associated with the development of autoimmunity. *ƒ* Human autoantibodies can be

directly pathogenic. *f* Immune complexes are often associated with systemic autoimmune disease. *f* Autoreactive B and T cells persist in normal subjects but in disease are selected by autoantigen in the production of autoimmune responses. *f* Microbial cross-reaching antigens and cytokine dysregulation can lead to autoimmunity. *f* Autoanitbody tests are valuable for diagnosis and sometimes for prognosis. *f* Treatment of organ-specific diseases usually involves metabolic control. *f* Treatment of systemic diseases includes the use of anti-inflammatory and immunosuppressive drugs. *f* Future treatment will probably focus on manipulation of the pivotal autoreactive T cells by antigens or peptides, by anti CD4 and possibly T cell vaccination.

THE SPECTRUM OF AUTOIMMUNE DISEASES

The antibodies associated with Hashimoto's thyroiditis and primary myxoedema react only with the thyroid, so the resulting lesion is highly localized. By contrast, the serum from patients with diseases such as systemic lupus crythematosus (SLE) reacts with many, if not all, of the tissues I the body. In SLE, one of the dominant antibodies is directed against the cell nucleus (Figure-2). These two diseases represent the extremes of the autoimmune spectrum (Figure-3). The common target organs in organ-specific disease include the thyroid, adrenals, stomach and pancreas. The non-organ-specific diseases, which include the rheumatological disorders, characteristically involve the skin, kidney, joints and muscle (Figure-4)

Human autoantibodies can be directly pathogenic

When investigating human autoimmunity directly, rather than using animal models, it is of course more difficult to carry out experiments. Nevertheless, there is much evidence to suggest that autoantibodies may be important in pathogenesis, and we will discuss the major examples here. Thyroid autoimmune disease – A number of disease have been recognized in which autoantibodies to hormone receptors may actually mimic the function of the normal hormone concerned and produce disease. Graves' disease (thyrotoxicosis) was the first disorder in which such antireceptor antibodies were clearly recognized. The phenomenon of neonatal thyrotoxicosis provides us with a natural 'passive transfer' study, because the IgG antibodies from the thyrotoxic mother cross the placenta and react directly with thyroid stimulating hormone (TSH) receptor o the neonatal thyroid. Many babies born to thyrotoxic mothers and showing thyroid hyperactivity have been reported, but the problem spontaneously resolves as the antibodies derived from the mother are catabolized in the baby over several weeks. Whereas autoantibodies to the TSH receptor may stimulate cell division and/or increase the production of thyroid hormones, others can bring about the opposite effect by inhibiting these functions, a

phenomenon frequently observed in the receptor responses to ligands which act as agonists or antagonists. Different combinations of the various manifestations of thyroid autoimmune disease, chronic inflammatory cell destruction and stimulation or inhibition of growth and thyroid hormone synthesis, can give rise to a wide spectrum of clinical thyroid dysfunction (Figure-9). Myasthenia gravis – A parallel with neonatal hyperthyroidism has been observed with mothers suffering from myasthenia gravis, where antibodies to acetylcholine receptors cross the placenta into the fetus and may cause transient muscle weakness in the newborn baby.

Pernicious anaemia –

In this disease an autoantibody interferes with the normal uptake of vitamin B12.Vitamine B12 is not absorbed directly, but must first associated with a protein called intrinsic factor; the vitamin-protein complex is then transported across the intestinal mucosa. Early passive transfer studies demonstrated that serum from a patient with pernicious anaemia, if fed to a healthy individual together with intrinsic factor - B12 complex, inhibited uptake of the vitamin. Subsequently, the factor in the serum which blocked vitamin uptake was identified as antibody against intrinsic factor. It is now known that plasma cells in the gastric mucosa of patients with pernicious anaemia secrete this antibody into the lumen of the stomach (Figure-10).

Goodpasture's syndrome –

In goodpasture's syndrome, antibodies to the glomerular capillary basement membrane bind to the kidney in vivo (Figure-3). To demonstrate that the antibodies can have a pathological effect, a passive transfer experiment was performed. The antibodies were eluted from the kidney of a patient who had died with this disease, and injected into primates whose kidney antigens were sufficiently similar for the injected antibodies to localize on the glomerular basement membrane. The injected monkeys subsequently died with glomerulonephritis.

Blood and vascular disorders –

Autoimmune haemolytic anaemia and idiopathic thrombocytopenic purpura result from the synthesis of autoantibodies to red cells and platelets, respectively. The primary antiphospholipid syndrome characterized by recurrent thromboembolic phenomena and feta loss is triggered by the reaction of autoantibodies with a complex of β2-glycoprotein turns up again as an abundant component of atherosclerotic plaques and there is increasing attention to the idea that autoimmunity may initiate or exacerbate the process of lipid deposition and plaque formation in this disease, the two lead candidate antigens being heat-shock protein 60 and the low-density lipoprotein, apoprotein B. The necrotizing granulomatous vasculitis which characterizes Wegener's granulomatosis is associated with anibodies to neutrophil cytoplasmic proteinase III (cANCA) but their role in pathogenesis of the vaculitis is ill defined.

pathogenic in systemic autoimmunity

In the case of SLE, it can be shown that complementfixing complexes of anibody with DNA and other nucleosome components such as histones are deposited in the kidney (Figure-3), skin, joints and choroid plexus of patients, and must be presumed to produce Type III hypersensitivity reactions. Cationic anti-DNA antibodies and histones facilitate the binding to heparin sulphate in the connective tissue structures. Individuals with genetic deficiency of the early classical pathway complement components clear circulating immune complexes very poorly and are unduly susceptible to the development of SLE.

DIAGONOSTIC AND PROGNOSTIC VALUE OF AUTOANTIBODIES

Wherever the relationship of autoantibodies to the disease process, they frequently provide valuable markers for diagnostic purposes. A particularly good example is the test for mitochondrial antibodies, used in diagnosing primary biliary cirrhosis (Figure-23). Exploratory laparotomy was previously needed to obtain this diagnosis, and was often hazardous because of the age and condition of the patients concerned. Autoantibodies often have predictive value. For instance, individuals testing positively for antibodies to both insulin and glutamic acid decarboxylase have a high risk of developing insulin-dependent diabetes.

TREATMENT

Often, in organ-specific autoimmune disorders, the symptoms can be conrolled by administration of thyroxine, and thyrotoxicosis by antithyroid drugs. In pernicious anaemia, metabolic correction is achieved by injection of vitamin B12, and in myasthenia gravis by administration of cholinesterase inhibitors. If the target organ is not completely destroyed, it may be possible to protect the surviving cells by transfection with FasL or TGFβ genes. Where function is completely lost and cannot be substituted by hormones, as many occur in lupus nephritis or chronic rheumatoid arthritis, tissue grafts or mechanical substitutes may be appropriate. In the case of tissue grafts, protection from the immunological processes which necessitated the transplant may be required.

Conventional immunosuppressive therapy with antimitotic drugs at high doses can be used to dam down the immune response but, because of the dangers involved, tends to be used only in life-threatening disorders such as SLE and dermatomyositis. The potential of cyclosporine and related drugs such as rapamycin has yet to be fully realized, but quite dramatic results have been reported in the treatment of type 1 diabetes mellitus. Anitinflammatory drugs are, of course, prescribed for rheumatoid diseases with the introduction of selective cyclo-oxygenase-2 (COX-2) inhibitors representing a welcome development. Encouraging results are being obtained by treatment of rheumatoid arthritis patients with low steroid doses at an early stage

to correct the apparently defective production of these corticosteroids by the adrenal feedback loop, and for those with more established disease, attention is now focused on the striking remissions achieved by synergistic treatment with anti-TNFα monoclonals plus methotrexate.

Immunological Tolerance

- Immunological tolerance is the failure to mount an immune response to an antigen. It can be:
- Natural or "self" tolerance. This is the failure (a good thing) to attack the body's own proteins and other antigens. If the immune system should respond to "self", an autoimmune disease may result.
- Induced tolerance. This is tolerance to external antigens that has been created by deliberately manipulating the immune system. Its importance:
  - to protect us from unpleasant, even dangerous, allergic reactions to such things as food (e.g. peanuts), insect stings, grass pollen (hay fever).
  - to enable transplanted organs (e.g., kidney, heart, liver) to survive in their new host; that is, to avoid graft rejection.
  - to reveal the mechanisms of autoimmunity in the hope of designing treatments for such diseases as systemic lupus erythematosus (SLE) and multiple sclerosis (MS).
- Immunological tolerance is not simply a failure to recognize an antigen; it is an active response to a particular epitope and is just as specific as an immune response.
- Both B cells and T cells can be made tolerant, but it is more important to tolerize T cells than B cells because B cells cannot make antibodies to most antigens without the help of T cells.

T-cell Tolerance Central Tolerance

In the thymus, the epitopes recognized by these receptors consist of:

- a small molecule, usually a peptide of 6–8 amino acids derived from body proteins; that is, "self" proteins nestled in [View]
- a histocompatibility molecule (encoded by the MHC)
  - class II for $CD4^+$ T cells
  - class I for $CD8^+$ T cells

T cells whose receptors bind these epitopes so tightly that they could attack the cell displaying them are deleted by apoptosis. The T cells that survive this negative selection leave the thymus and migrate throughout the immune system (lymph nodes, spleen, etc.).

The antigen-presenting cells in the thymus are certainly capable of presenting peptide fragments from the many "housekeeping" proteins found in all cells (e.g., the enzymes used in

glycolysis). But there are many proteins that are expressed only in differentiated cells that are restricted to a particular tissue e.g., the insulin-producing beta cells in the islets of Langerhans in the pancreas. How is central tolerance to these proteins achieved in the thymus?

It turns out that antigen-presenting cells in the medulla of the thymus express a gene, *AIRE*, that encodes a transcription factor that turns on the expression of hundreds of tissue- specific genes encoding such proteins as

- the precursor to insulin
- thyroglobulin (precursor of the thyroxine secreted by the thyroid gland)
- casein (protein in the milk secreted by the mammary glands) a protein secreted by the salivary glands

• The AIRE protein does not seem to increase the expression of housekeeping genes. How it distinguishes between these and the tissue-specific genes to be turned on remains to be discovered.

• AIRE protein binds to chromatin whose histone H3 has no methyl groups attached to its lysine-4 ("$H3K4me^0$". This is a mark of inactive genes.

• AIRE stands for autoimmune regulator. Knockout mice and those rare humans who have no functioning *AIRE* gene suffer from severe autoimmune disease especially of their various endocrine organs.

Peripheral Tolerance

• The T cells that leave the thymus are relatively — but not completely — safe. Some will have receptors (TCRs) that can respond to self antigens

- that are present in such high concentration that they can bind to "weak" receptors;
- that they may not have encountered in the thymus. Thanks to the activity of AIRE, the list of the latter molecules

Five possibilities for which there is substantial evidence:

Negative Selection in the Peripheral Immune System

AIRE is also active in some antigen-presenting cells in the organs of the peripheral immune system, e.g., lymph nodes and spleen. So any potentially autoreactive T cells that failed to be eliminated in the thymus can be selected against in these tissues. The binding of a T cell to an antigen-presenting cell (APC) is by itself not enough to activate the T cell and turn it into an effector cell: one able to, for examples, kill the APC ($CD8^+$ cytotoxic T lymphocytes [CTLs])

carry out cell-mediated immune reactions ($CD4^+$ Th1 cells) provide help to B cells ($CD4^+$ Th2 cells) may not be as large as we once thought.

Although T cells encounter self antigens in body tissues, they will not respond unless they receive a second signal. In fact, binding of their TCR ("signal one") without "signal two" causes them to self-destruct by apoptosis. Most of the time, the cells presenting the body's own antigens either fail to provide signal two or transmit an as-yet-unidentified second signal that turns the T cell into a regulatory T cell (Treg) that suppresses immune responses.

In either case, self-tolerance results

1. Failure to Encounter Self Antigens

- Some tissues are hidden behind anatomical barriers that keep T cells from reaching them. Examples of such "privileged sites":
  - interior of the eye
  - testes
  - the brain
- Mechanical damage can breach the barrier and an autoimmune attack follow.

2. Receipt of Death Signals

- Some cells of the body express the Fas ligand, FasL. Activated T cells always express Fas. When they encounter these cells, binding of Fas to FasL triggers their death by apoptosis.

Examples:

- Cells within the eye always express FasL and are thus ready to kill off any rogue T cells that might gain entry.

Macrophages infected with HIV express FasL and thus kill any anti-HIV T cells that try to kill them. This may account for the disastrous decline in $CD4^+$ T cells late in the development of AIDS.

3. Control by Regulatory T Cells

- A minor population of $CD4^+$ T cells, called regulatory T cells, suppresses the activity of other T cells. They may be important players in protecting the body from attack by its other T cells.

B-cell Tolerance

- The problem of B-cell tolerance is not so acute because B cells cannot respond to most antigens unless they receive help from T helper cells.
- Nevertheless, B cells become tolerized to self components and, like T cells, this occurs both in the bone marrow (central tolerance) and elsewhere in the body

(peripheral tolerance).

Central Tolerance

- B cells are formed and mature in the bone marrow. In humans, over half of the developing B cells produce a BCR able to bind self components.
- Any cells that produce a receptor for antigen (BCR) that would bind self components too tightly undergo a process of receptor editing. They dip again into their pool of gene segments that encode the light and heavy chains of their BCR and try to make a new BCR that is not a threat. If they fail, they commit suicide (apoptosis).
- Despite these mechanisms, some of the B cells that migrate out of the bone marrow continue to express self-reactive BCRs and may still be able to produce anti-self antibodies. So a mechanism is needed to tolerize them out in the tissues ("peripheral tolerance").

Peripheral Tolerance

- B cells with a potential for attacking self can be kept in check by the absence of the T-helper cells they need; that is, T-cell tolerance is probably the most important (but not the only) mechanism for maintaining B-cell tolerance.

Induced Tolerance Allergies

Allergists have struggled for years to find safe ways to tolerize allergic people to their allergens. This has usually involved giving a long series of injections of a special formulation of the allergen.

Examples:

- the active ingredient in poison ivy that triggers this cell-mediated immune response;
- allergens that trigger IgE-mediated allergic responses, such as
  - ragweed, grass, and tree pollens;
  - insect stings;
  - food allergens, e.g., peanuts and other nuts

Transplant Tolerance

If ways could be found to induce genuine tolerance to allografts (organs transplanted from another person), this would enable the organ to resist rejection without the need for continuous use of immunosuppressive drugs.

It also has inspired attempts to achieve graft tolerance in humans by pretreating the recipient with blood (rich in B cells) or bone marrow of the donor.

In such cases (as well as Billingham's), it may be that tolerance of the graft is created because

the priming cells are unable to give a second signal to host T cells and maintained by the continued survival in the recipient of these donor cells.

Tolerance of the Fetus

The human foetus is also an allograft, but the mother makes no attempt to reject it (at least for 9 months). How this tolerance is established and maintained is still being studied.

HIV/AIDS

The first cases of acquired immunodeficiency syndrome (AIDS) were reported in the United States in the spring of 1981. By 1983 the human immunodeficiency virus (HIV), the virus that causes AIDS, had been isolated. The largest group of early AIDS cases comprised gay and bisexual men (referred to as men who have sex with men. Early cases of HIV infection that were sexually transmitted often were related to the use of alcohol and other substances, and the majority of these cases occurred in urban, educated, white MSMs. HIV/AIDS prevalence rates among injection drug users vary by geographic region, with the highest rates in surveyed substance abuse treatment centres in the Northeast, the South, and Puerto Rico.

The virus is transmitted primarily through the exchange of blood using needles, syringes, that were previously used by an HIV-infected person. Another route of HIV transmission among injection drug users is through sexual contacts within relatively closed sexual networks, which are characterized by multiple sex partners, unprotected sexual intercourse, and exchange of sex for money. The inclusion of alcohol and other noninjection substances to this lethal mixture only increases the HIV/AIDS caseload. A major risk factor for HIV/AIDS among injection drug users is crack use; one study found that crack abusers reported more sexual partners in the last 12 months, more sexually transmitted diseases (STDs) in their lifetimes, and greater frequency of paying for sex, exchanging sex for drugs, and having sex with injection drug users.

Following are the key concepts about HIV/AIDS

Substance abuse increases the risk of contracting HIV. HIV infection is substantially associated with the use of contaminated or used needles to inject heroin. Also, substance abusers may put themselves at risk for HIV infection by engaging in risky sex behaviors in exchange for powder or crack cocaine. However, this fact does not minimize the impact of other substances that may be used

Substance abusers are at risk for HIV infection through sexual behaviors. Both men and women may engage in risky sexual behaviors for the purpose of obtaining substances, while under the influence of substances, or while under coercion.

Substance abuse treatment serves as HIV prevention. Placing the client in substance abuse treatment along a continuum of care and treatment helps minimize continued risky substance-abusing practices. Reducing a client's involvement in substance-abusing practices reduces the probability of infection.

HIV/AIDS, substance abuse disorders, and mental disorders interact in a complex fashion. Each acts as a potential catalyst or obstacle in the treatment of the other two--substance abuse can negatively affect adherence to HIV/AIDS treatment regimens; substance abuse disorders and HIV/AIDS are intertwining disorders; HIV/AIDS is changing the shape

and face of substance abuse treatment; complex and legal issues arise when treating HIV/AIDS and substance abuse; HIV-infected women with substance abuse disorders have special needs.

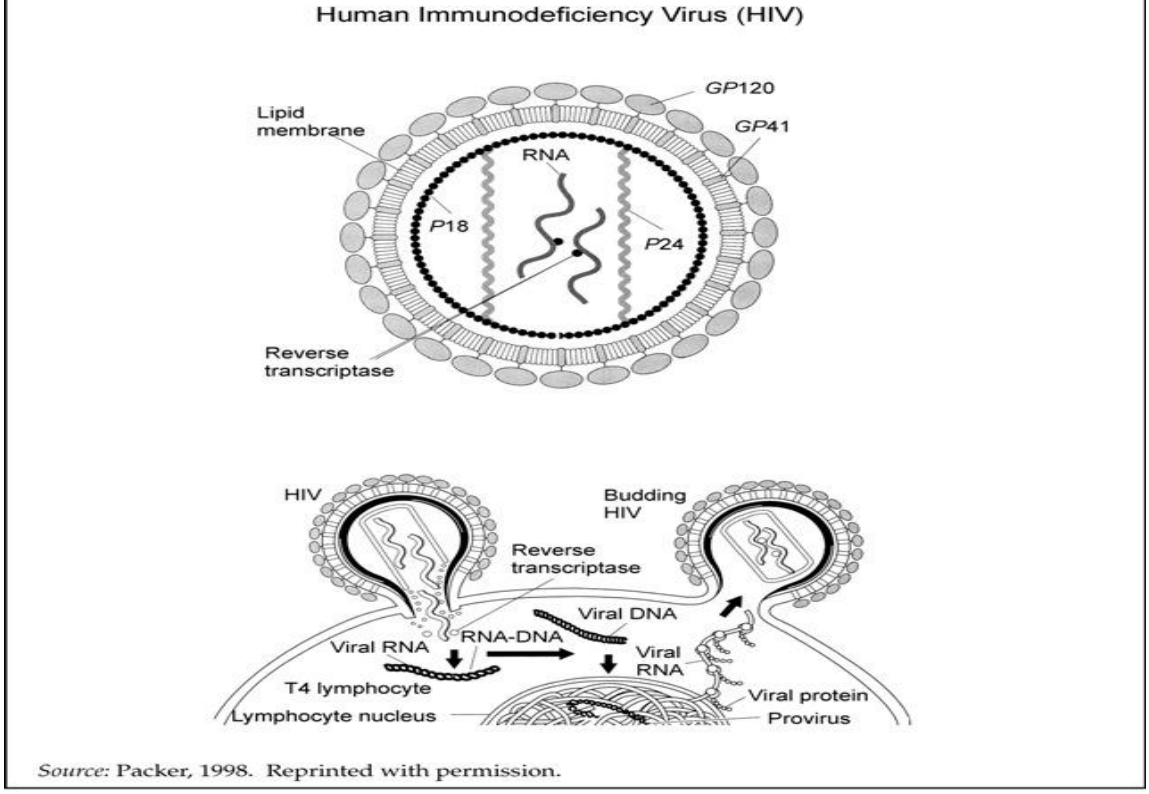
Risk reduction allows for a comprehensive approach to HIV/AIDS prevention. This strategy promotes changing substance-related and sex-related behaviors to reduce clients' risk of contracting or transmitting HIV.

Origin of HIV/AIDS

Many theories and myths about the origin of HIV, the most likely explanation is that HIV was introduced to humans from monkeys. A recent study identified a subspecies of chimpanzees native to west equatorial Africa as the original source of HIV-1, the virus responsible for the global AIDS pandemic. Monkeys can carry a virus similar to HIV, known as SIV (simian immunodeficiency virus), and there is strong evidence that HIV and SIV are closely related. AIDS is caused by HIV infection and is characterized by a severe reduction in CD4+ T cells, which means an infected person develops a very weak immune system and becomes vulnerable to contracting life-threatening infections such as Pneumocystis carinii pneumonia.

HIV/AIDS is still largely a disease of MSMs and male injection drug users, but it is spreading most rapidly among women and adolescents, particularly in African American and Hispanic communities. HIV is a virus that thrives in certain ecological conditions. The following will lead to higher infection rates: a more potent virus, high viral load, high prevalence of STDs, substance abuse, high HIV seroprevalence within the community, high rate of unprotected sexual contact with multiple partners, and low access to health care. These ecological conditions exist to a large degree among urban, poor, and marginalized communities of injection drug users.

Figure 1-1
Parts of HIV



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HIV Transmission

HIV cannot survive outside of a human cell. HIV must be transmitted directly from one person to another through human body fluids that contain HIV-infected cells, such as blood, semen, vaginal secretions, or breast milk. The most effective means of transmitting HIV is by direct contact between the infected blood of one person and the blood supply of another. This can occur in childbirth as well as through blood transfusions or organ transplants prior to 1985. Using injection equipment that an infected person used is another direct way to transmit HIV.

Sexual contact is also an effective transmission route for HIV because the tissues of the anus, rectum, and vagina are mucosal surfaces that can contain infected human body fluids and because these surfaces can be easily injured, allowing the virus to enter the body. A person is about five times more likely to contract HIV through anal intercourse than through vaginal intercourse because the tissues of the anal region are more prone to breaks and bleeding during sexual activity.

A woman is eight times more likely to contract HIV through vaginal intercourse if the man is infected than in the reverse situation. HIV can be passed from a woman to a man during intercourse, but this is less likely because the skin of the penis is not as easily damaged. Female-to-female transmission of HIV apparently is rare but should be considered a possible means of transmission because of the potential exposure of mucous membranes to vaginal secretions and menstrual blood.

Oral intercourse also is a potential risk but is less likely to transmit the disease than anal or vaginal intercourse. Saliva seems to have some effect in helping prevent transmission of HIV, and the oral tissues are less likely to be injured in sexual activity than those of the vagina or anus.

Life Cycle of HIV

It is possible to prevent transmission even after exposure to HIV. In San Francisco, postexposure prophylaxis is being offered to people who believe they have high risk for HIV transmission because of exposure with a known or suspected HIV-infected individual. Treatment is started within 72 hours of exposure and includes combination therapy, which may include a protease inhibitor, for a period of 1 month and follow up for 12 months.

Once HIV particle enters a person's body, it binds to the surface of a target cell (CD4+ T cell). The virus enters through the cell's outer envelope by shedding its own viral envelope, allowing the HIV particle to release an HIV ribonucleic acid (RNA) chain into the cell, which is then converted into deoxyribonucleic acid (DNA). The HIV DNA enters the cell's nucleus and is copied onto the cell's chromosomes. This causes the cell to begin reproducing more HIV, and eventually the cell releases more HIV particles. These new particles then attach to other target cells, which become infected.

Measuring HIV in the blood

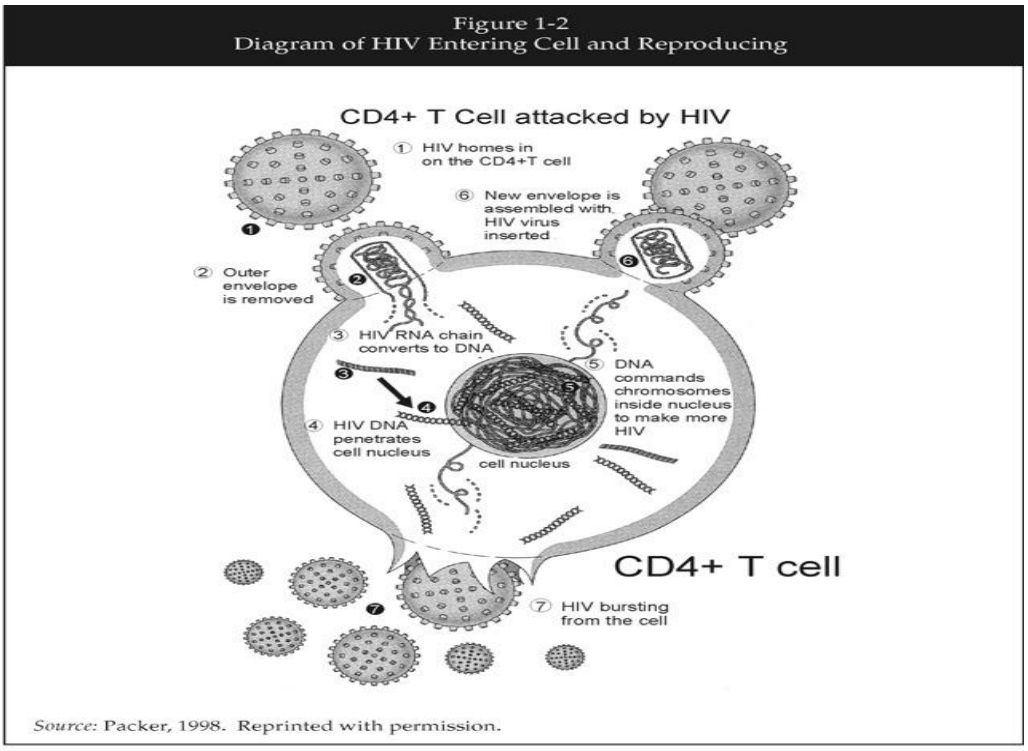
Physicians can measure the presence of HIV in a person by means of (1) the CD4+ T cell count and (2) the viral load count. The CD4+ T cell count measures the number of CD4+ T cells in a millilitre of blood. These are the cells that HIV is most likely to infect, and the number of these cells reflects the overall health of a person's immune system. CD4+ T cells act as signals to inform the body's immune system that an infection exists and needs to be fought. Because HIV hides inside the very cells responsible for signalling its presence, it can survive and reproduce without the infected person knowing of its existence for many years. Even though the body can produce sufficient CD4+ T cells to replace the billions that are destroyed by untreated HIV

each day, eventually HIV kills so many CD4+ T cells that the damaged immune system cannot control other infections that may make the person sick. This is the late stage of HIV, when AIDS is often diagnosed based on the presence of specific illnesses (i.e., opportunistic infections).

The viral load represents the level of HIV RNA circulating in the bloodstream. This level becomes very high soon after a person is initially infected with HIV, then it drops. Viral load tests measure the number of copies of the virus in a milliliter of plasma; currently available tests can measure down to 50 copies per milliliter, and even more sensitive tests can measure down to 5 copies per milliliter.

The 6 to 12 weeks between the time of infection and the time when an ELISA test for HIV becomes positive are called the "window period." During this period, the individual is extremely infectious to any sexual or needle-sharing partner but does not test positive unless a more expensive viral load test is performed.

Figure 1-2
Diagram of HIV Entering Cell and Reproducing



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Initial infection

Primary HIV infection can cause an acute retroviral syndrome that often is mistaken for influenza (the flu), mononucleosis, or a bad cold. This syndrome is reported by roughly half of those who contract HIV and generally occurs between 2 and 6 weeks after infection. Symptoms may include fever, headache, sore throat, fatigue, body aches, weight loss, and swollen lymph nodes. Other symptoms are a rash, mouth or genital ulcers, diarrhea, nausea and vomiting, and thrush. The CD4+ T cell count can drop very low during the early weeks, although it usually returns to a normal level after the initial illness is over. The initial illness can last several days or even weeks.

The greatest spread of HIV occurs throughout the body early in the disease. Approximately 6 months after infection, the level of virions produced every day may reach a "set point." A higher set point usually means a more rapid progression of HIV disease. Early treatment may be

recommended to reduce the set point, potentially leading to a better chance of controlling the infection.

Latency period

After initial infection comes the latency period, or incubation period, during which untreated persons with HIV have few, if any, symptoms. This period lasts a median of about 10 years. The most common symptom during this period is lymphadenopathy, or swollen lymph nodes. The lymph nodes found around the neck and under the arms contain cells that fight infections. Swollen lymph nodes in the groin area may be normal and not indicative of HIV. When any infection is present, lymph nodes often swell, sometimes painfully. With HIV, they swell and tend to stay swollen but usually are not painful.

Early symptomatic infection

After the first year of infection, the CD4+ T cell count drops at a rate of about 30 to 90 cells per year. When the CD4+ T cell count falls below 500, mild HIV symptoms may occur. Many people, however, will have no symptoms at all until the CD4+ T cell count has dropped very low (200 or less). Bacteria, viruses, and fungi that normally live on and in the human body begin to cause diseases that are also known as opportunistic infections. Early symptoms of infection may include chronic diarrhea, herpes zoster, recurrent vaginal candidiasis, thrush, oral hairy leukoplakia (a virus that causes white patches in the mouth), abnormal Pap tests, thrombocytopenia, or numbness or tingling in the toes or fingers. Most of these infections occur with a CD4+ T cell count between 200 and 500. Symptoms of these infections usually signal a problem with the immune system but are not severe enough to be classified as AIDS.

AIDS

In the 1980s, AIDS was defined to include a depressed immune system and at least one illness tied to HIV infection. AIDS-defining conditions are diseases not normally manifest in someone with a healthy immune system. These should prompt a confirmatory HIV test. The additional 1993 AIDS-defining conditions led to the diagnosis of more AIDS cases in women and injection drug users. Since 1993, the list of AIDS-defining conditions has included pulmonary tuberculosis (TB), recurrent bacterial pneumonia, and invasive cervical cancer. HIV-infected persons with a CD4+ T cell count of 200 or less are classified as persons with AIDS.

TB and invasive cervical cancer are two AIDS-defining conditions that warrant special mention. Pulmonary TB is the one AIDS-related infection that is contagious to those without HIV. It generally causes a chronic dry cough, fatigue, and weight loss. Cervical cancer may progress rapidly in women with HIV but usually is asymptomatic until it is too late for successful treatment. Women who are HIV positive should have Pap tests at least once every 6 months and more often if any abnormality is found.

AIDS symptoms

Most AIDS-defining diseases are severe enough to require medical care, sometimes hospitalization. Cough is a symptom common to several AIDS-related infections, the most frequent of which is *Pneumocystis carinii* pneumonia. PCP is characterized by a dry cough, fever, night sweats, and increasing shortness of breath. Recurrent bacterial pneumonia also is an AIDS-defining condition. Several skin problems can occur in HIV/AIDS. Kaposi's sarcoma (KS), a rare malignancy outside of HIV disease, may be the best-known skin condition in HIV infection. KS is a cancer of the blood vessels that causes pink, purple, or brown splotches, which appear usually as firm areas on or under the skin. KS also grows in other places, such as the lungs and mouth. KS is highly prevalent among men with AIDS, of whom 20 to 30 percent may develop the condition in contrast to 1 to 3 percent of women with AIDS.

Diarrhea is a very common symptom of AIDS. Many AIDS-defining conditions cause diarrhea, including parasitic, viral, and bacterial infections. HIV itself can cause diarrhea if it infects the intestinal tract. Diarrhea also is a common side effect of HIV/AIDS medications. Weight loss

can be caused by inadequate nutrition, untreated neoplasms and opportunistic infections, and deranged metabolism.

Changes in vision, particularly spots or flashes, may indicate an infection inside the eye. A virus called cytomegalovirus (CMV) is the most common cause of blindness in people with HIV/AIDS. CMV progresses very rapidly if not treated and is among the most feared of AIDS-related infections. A severe headache, seizure, or changes in cognitive function may herald the onset of a number of infections or cancers inside the brain. The two most common brain infections in HIV/AIDS are cryptococcal meningitis, a fungus that usually causes a severe headache, and toxoplasmosis, which can present with focal neurologic deficits or seizure. Seizures also can be caused by the cancer of the central nervous system called lymphoma.

End-stage disease

A person with HIV/AIDS can live an active and productive life, even with a CD4+ T cell count of zero, if infections and cancers are controlled or prevented. The newer antiviral medicines can even help the body restore much of its lost immune function. In the past few years, a phenomenon called the Lazarus syndrome has developed among patients with AIDS, wherein, because of optimal drug therapy, someone who had seemed very near death improves and returns to fairly normal function. Untreated, the disease eventually overwhelms the immune system, allowing one debilitating infection after another. Sometimes the possible combinations of medication are no longer effective, the side effects are intolerable, or no further therapy is available.

Treatment

While there is no cure for HIV, treatments can stop the progression of the infection. Receiving these treatments, called antiretrovirals, can reduce the risk of transmission. It can also extend a person's life expectancy and improve the quality of life. Many people who take HIV treatments live long, healthy lives. These medications are becoming increasingly effective and well-tolerated. A person may need to take just one pill per day.

The following sections look at HIV treatments and medications for prevention.

Protease inhibitors

Integrase inhibitors

Nucleoside and nucleotide reverse transcriptase inhibitors

Chemokine coreceptor antagonists