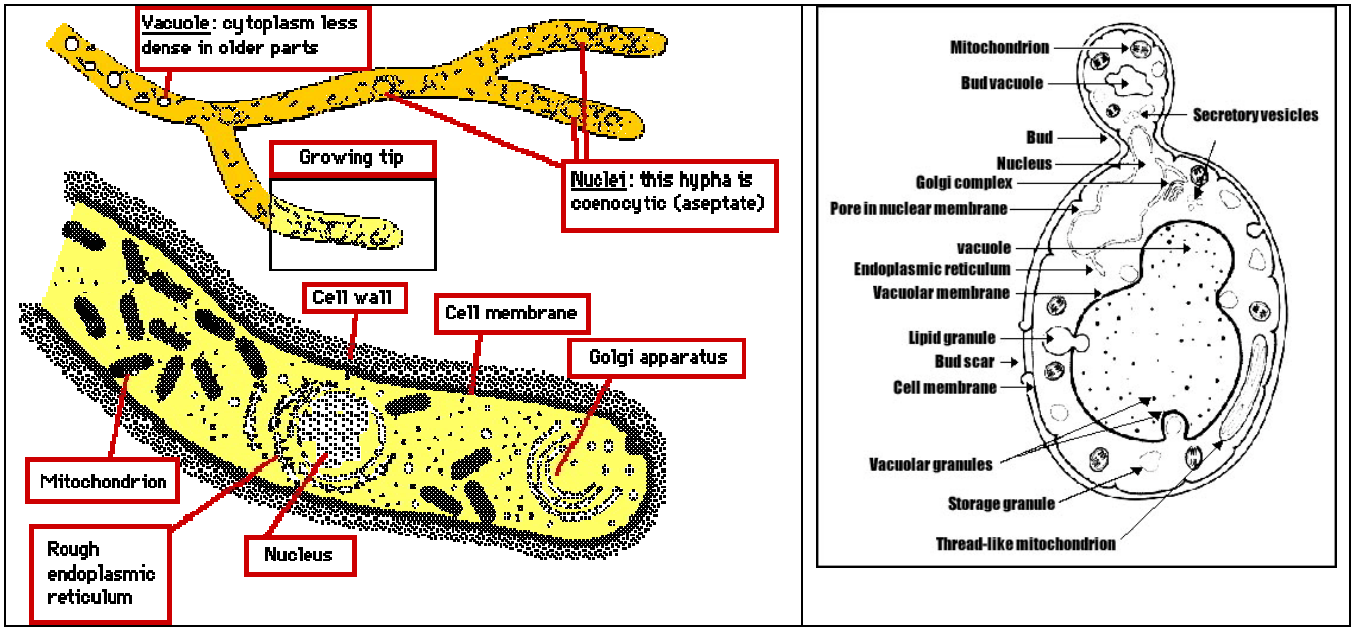
As we know **Medical mycology** A branch of medical microbiology that deals with the study of fungi which affect human health, especially those which produce disease (*mycosis*) , Its also include the study of fungal pathogenesis and epidemiology and its related to the study of disease pathology and immunology.

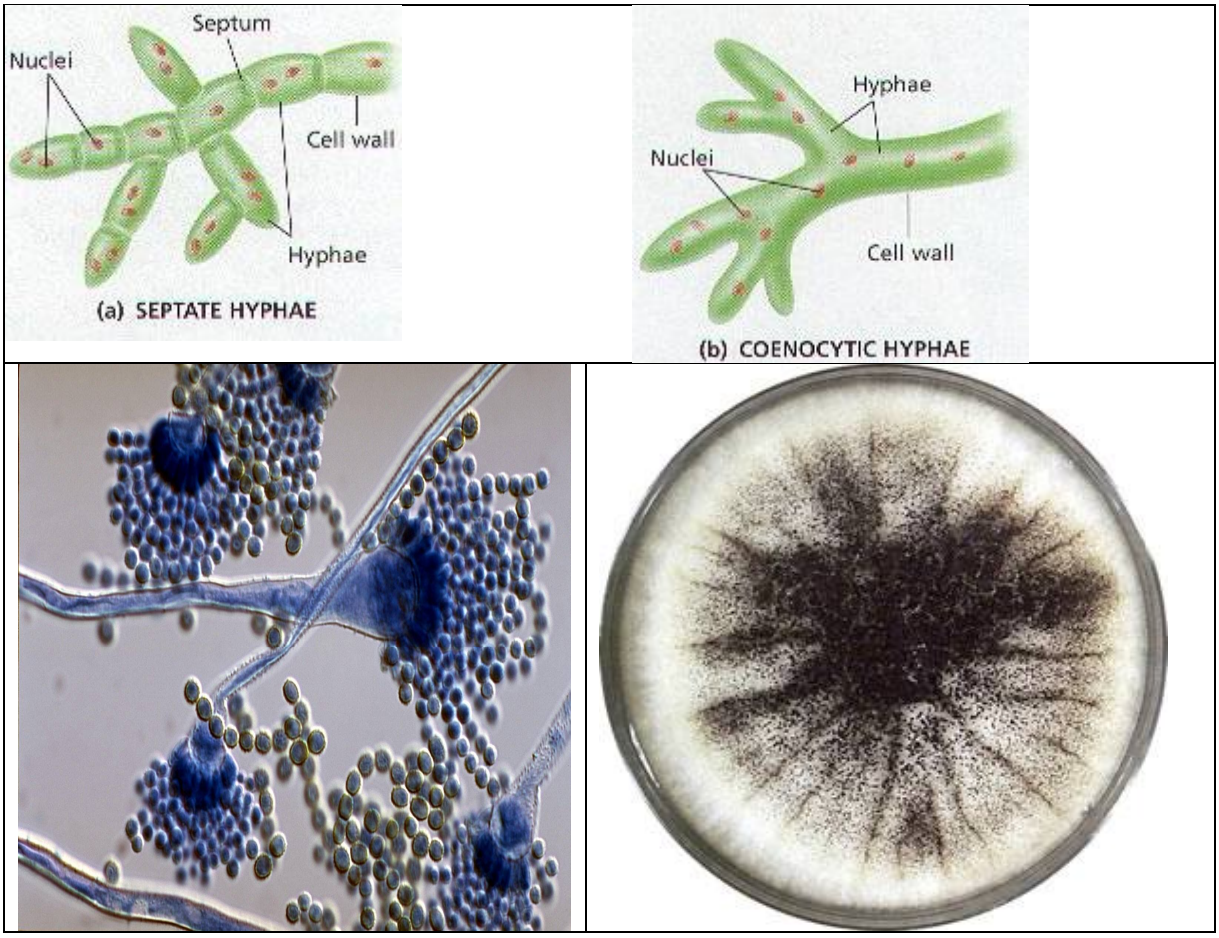
• Introduction

Fungi constitute a large, different group of heterotrophic organisms, most of which are found as saprophytes in the soil and on decomposing organic matter. They are eukaryotic with a range of internal membrane systems, organelles, and a well defined cell wall composed largely of polysaccharides (glucan, mannan) and chitin. they show large variation in size and form.

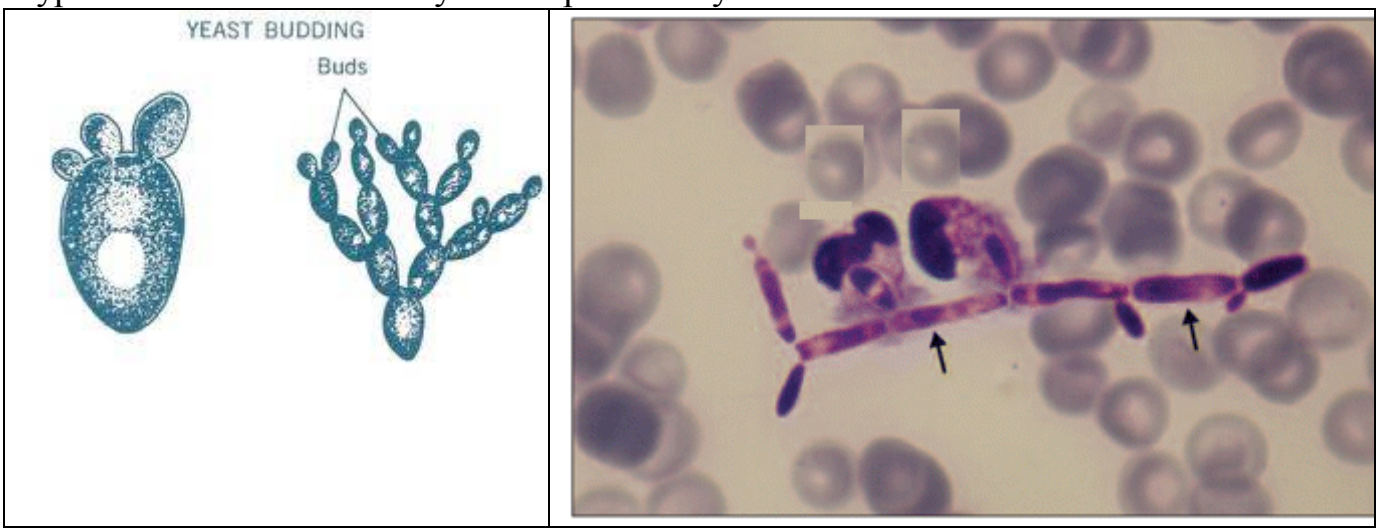


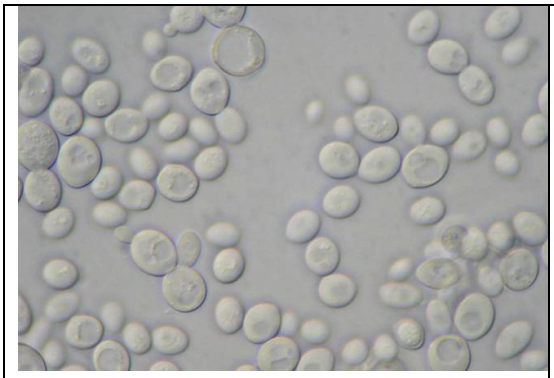
but we can be divided fungi into 3 main groups accrding to their forms :

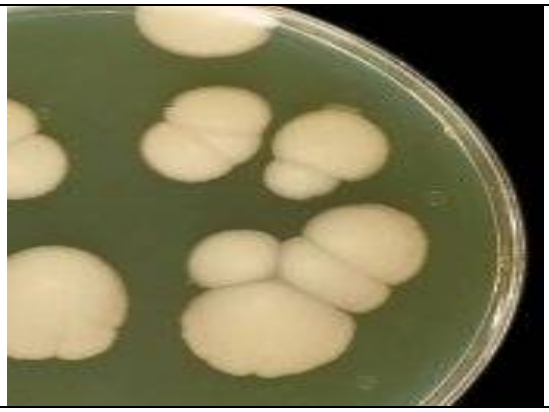
✹**Moulds**(multicellular filamentous fungi) which are composed of branching filaments termed hyphae that grow by apical extension to form a mass termed a mycelium . in most Fungi the hyphae have regular cross- wall(septa) but in lower fungi these are usually absent. Moulds reproduce by means of spores produced. Often in large numbers by an asexual process (involving mitosis only) or as a result of sexual reproduction (involving meiosis) . many fungi can produce more than one type of spore depending on the growth condition . in some higher fungi the sexual spores are produced in macroscopic structures such as mushrooms , in laboratory cultures moulds produce mainly asexual spores.



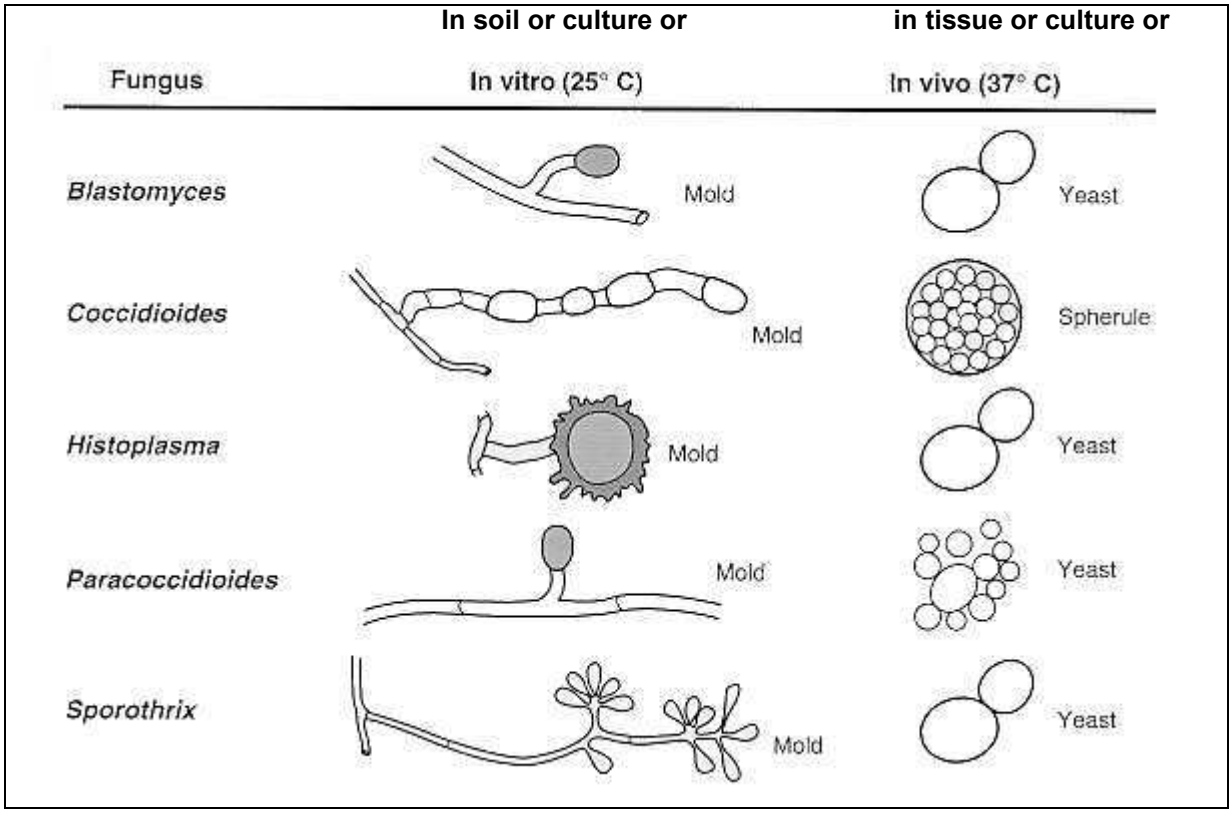
✹**Yeasts** , which are mostly unicellular and oval or round in shape. Most propagate by an asexual process called budding , some yeasts produce chains of elongated cell called pseudohyphae that look like the hyphae of moulds , but some species produce true hyphae. A small number of yeasts reproduce by fission.

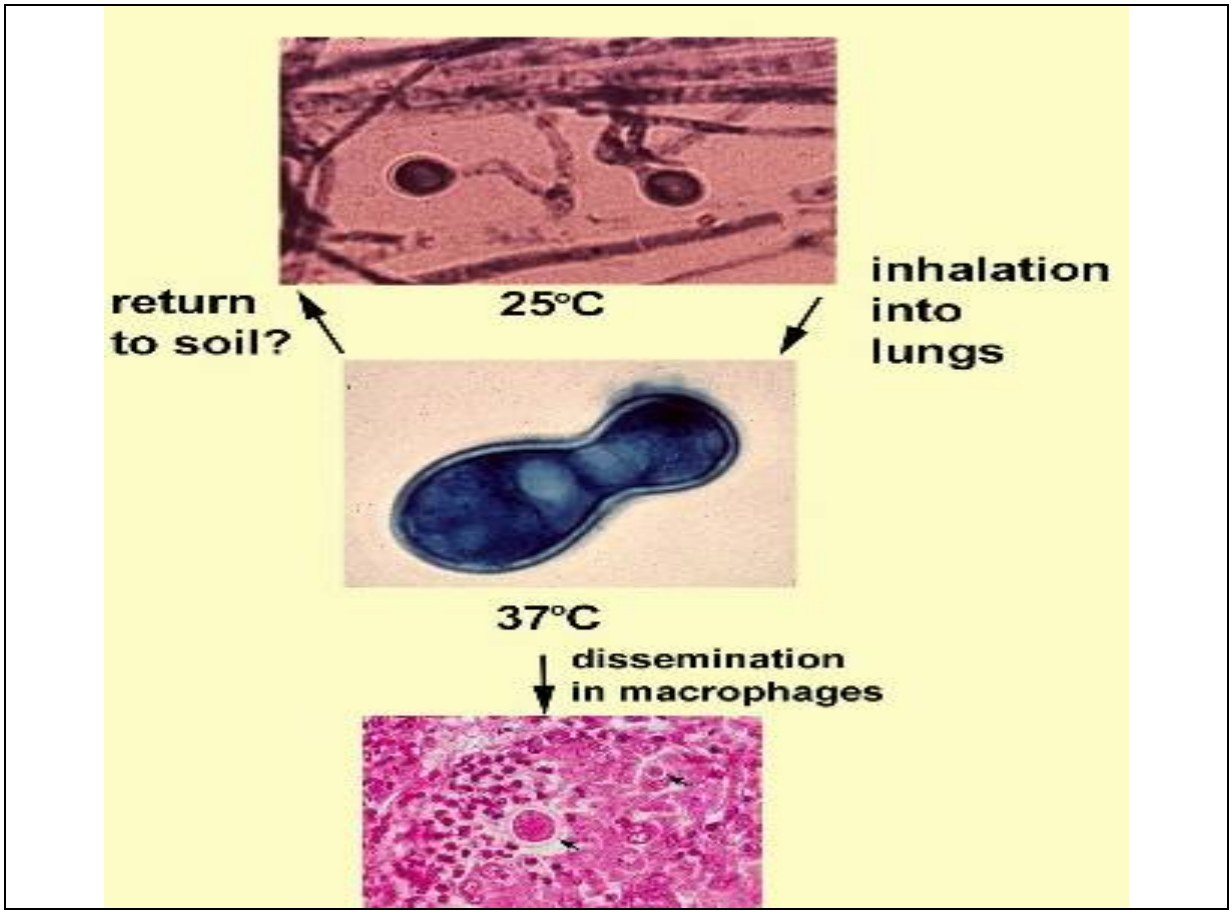






✹**Dimorphic fungi** which are able of changing their growth to either a mycelial or yeast phase depending on the growth conditions (natural environment, laboratory, tissue). Such as pathogenic fungi in the table below:





Fungal pathogenicity

☆Fungal pathogens

There are at least 100000 named species of fungi , fewer than 500 have been recognized causing disease (**mycosis**) in man or animals .in which the form of mycoses and severity of the infection depend on the degree of exposure to the fungus, the site and method of entry into the body , and the level of immunocompetence of the host.

Some fungi may cause serious , occasionally fatal, toxic effects in man , either following ingestion of poisonous mouldy food that contains toxic metabolites that known as mycotoxins. Allergic disease of the airways may also result from inhalation of fungal spores.

Usually Humans have a high level of innate immunity to fungi and most of the infections they cause are mild and self-limiting. This resistance is due to:

- 1. the fatty acid content of the skin,
- 2. the pH of the skin, mucosal surfaces and body fluids,
- 3. Epithelial cell turnover,
- 4. Normal flora,
- 5. Transferrin,
- 6. Cilia of the respiratory tract.

When fungi do pass the resistance barriers of the human body and establish infections, the infections are classified according to the tissue levels initially colonized.

☆**Fungal virulence factors** can be divided into 2 categories : virulence factors that encourage fungal colonization of the host and virulence factors that damage the host .

①**the virulence factors that encourage fungal colonization of the host include the ability to :**
contact host cells.
Adhere to host cells and resist physical removal.
Invade host cells.
Compete for nutrient.
Resist innate immune defenses such as phagocytosis and complement.
avoid immune defenses.

②**virulence factors that damage the host** –as fungi grow in the body they can secrete enzymes to digest cells these include protease, phospholipases ,elastases . in respose to both the fungus and to cell injury- cytokines are released , This leads to an inflammatory response and extracellular killing by phagocytes that causes more destruction of host tissues.
Also, Many molds secrete mycotoxins such as aflatoxins produced by certain *Aspergillus* species ……etc which causing a variety of effects in humans if ingested including : loss of muscle organization, weight loss, and tremors. In addition to that Some mycotoxins are mutagenic and carcinogenic .

☆**Types of fungal infections**

In Generaly As we know There are many types of mycotic diseases:

- Hypersensitivity - an allergic reaction to molds and spores.
- Mycotoxicoses - poisoning of man and animals by food products contaminated by fungi which produce toxins from the grain substrate e.g. aflatoxins produced by *Aspergillus flavus*.
- Fungal Infection - tissue invasion with a host response

So We shall be concerned only in the last type: **Fungal diseases** (**MYCOSES**) that caused by pathogenic fungi. The most practical method for medical students to discussed this type of infection is the clinical taxonomy which divides the Fungal diseases into:

- **Superficial mycoses**
- **Cutaneous mycoses**
- **Subcutaneous mycoses**
- **Dimorphic Systemic mycoses**

- **Opportunistic Systemic mycoses**

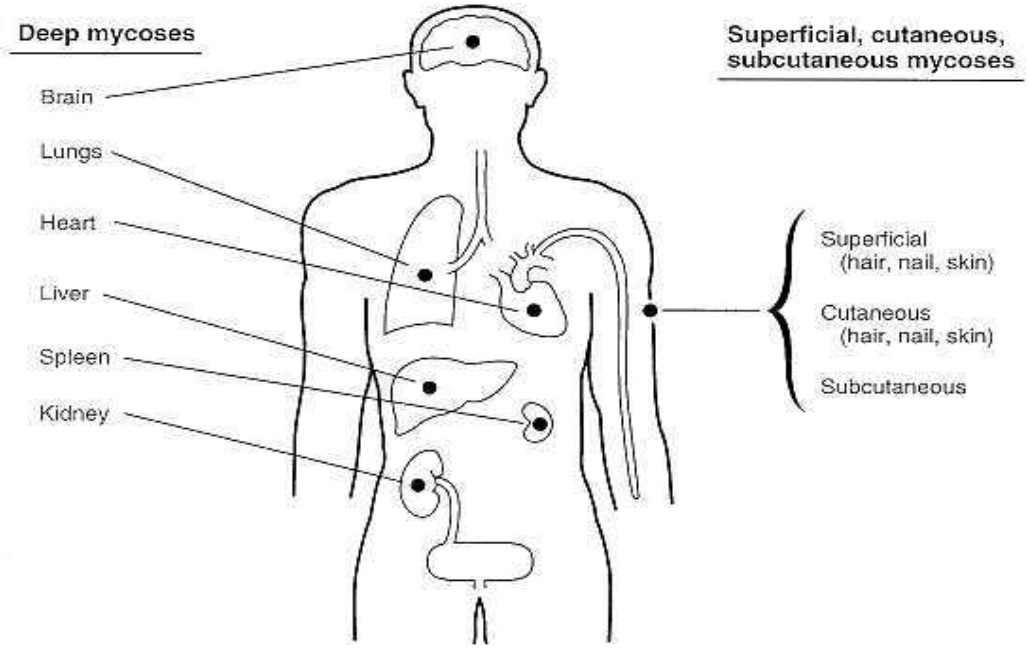


Figure: Principal tissue sites of deep mycoses in comparison to those of the superficial, cutaneous, and subcutaneous mycoses.

▓ Superficial mycoses :

Superficial mycoses are fungal infections of the skin and hair that invade only the most superficial layers and **cause little or no inflammatory response.** Such as :

Tinea nigra , **White piedra** , **Pityriasis versicolor** , **Black piedra** , **Otomycosis** , **Keratomycosis**

▓ Cutaneous mycoses :

Cutaneous mycoses extend deeper into the epidermis, and also include invasive hair and nail diseases. These diseases are restricted to the keratinized layers of the skin, hair, and nails. Unlike the superficial mycoses, host immune responses may be stimulat, resulting in pathologic changes expressed in the deeper layers of the skin. Such as :

Dermatophytosis (Tinea= Ring Worm) , **Candidiasis** , **Dermatomycosis**

▓ Subcutaneous mycoses :

Subcutaneous mycoses involve the dermis, subcutaneous tissues, muscle, and fasciaرباط . These infections are chronic and can be initiated by piercingنافذ trauma to the skin, which allows the fungi to enter. Such as :

Sporotrichosis , **Chromomycosis** , **Mycetoma** , **Lobomycosis** , **Hyphomycosis** ,……etc

▒ Opportunistic Systemic mycoses:

infections of patients with immune deficiencies (which include AIDS, alteration of normal flora by antibiotics, immunosuppressive therapy, ….) who would otherwise not be infected in the normal condition . Such as :

Cryptococcosis , Aspergillosis , Candidiasis , Other: Fusariosis , Penicillosis , ……..Any Fungus found in nature may give rise to OPPORTUNISTIC MYCOSES

▒ Dimorphic Systemic mycoses :

Dimorphic Systemic mycoses are fungal infections caused by dimorphic fungal (pathogens which can overcome the physiological and cellular defences of the normal human host by changing their morphological form) originate primarily in the lungs and may spread to many organ systems. Organisms that cause systemic mycoses are inherently virulent. Such as :

Histoplasmosis , Coccidioidomycosis , Blastomycosis , Paracoccidioidomycosis

☆Mechanisms of fungal disease

Encounter

With some exceptions, fungi concerned in human diseases are free- living in nature. Most mycoses are acquired as a result of accidental encounters by inhalation or traumatic implantation from an exogenous source.

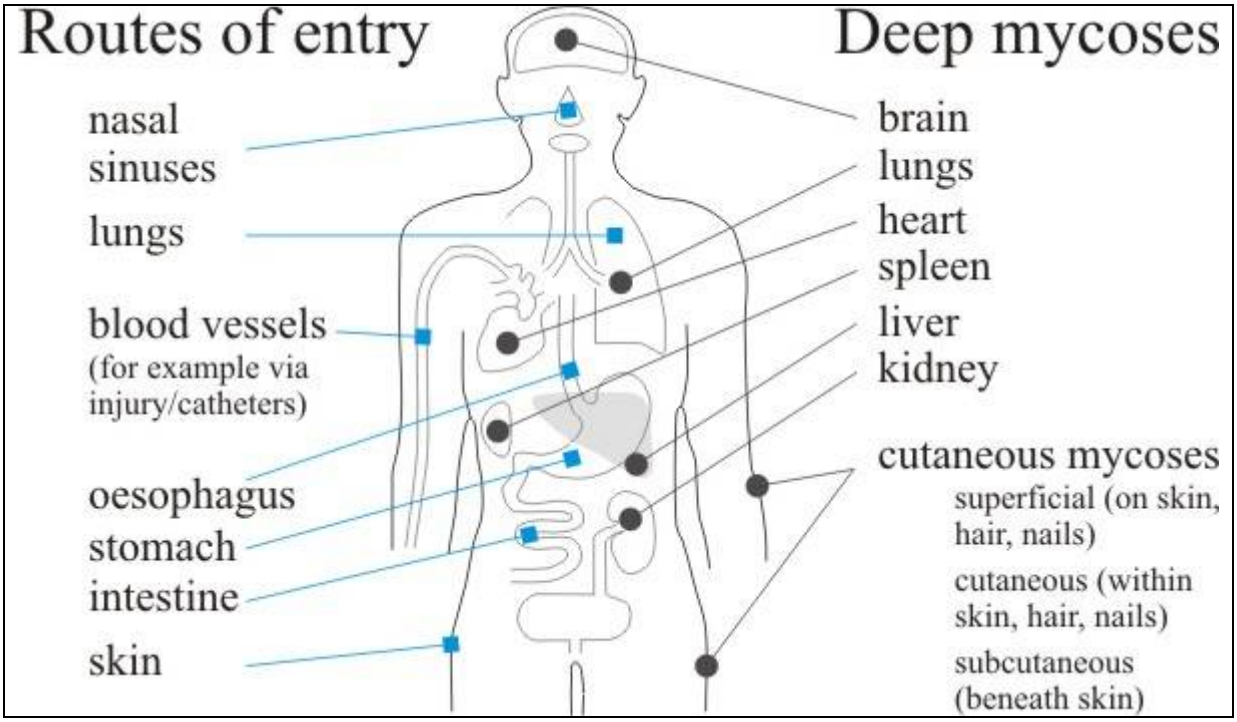
For example, *H. capsulatum* is found in soil contaminated by the excreta of bats, chickens and starlings زرازير. *C. neoformans* is associated with pigeon roosts اعشاش and soils contaminated by pigeon droppings.

In contrast to these environmental habitats, many of us carry *C.albicans* in the mouth, gastrointestinal tract , and other mucous membrane as part of our normal flora . also there is *Malassezia furfur* a yeasts found on the healthy human skin , particularly in the upper trunk, face and scalp, the areas that are rich in sebaceous glands, which produce lipids used by these organisms.

Entry

The level of innate immunity to pathogenic fungi is high in most humans, as the fact says that fungal infections are usually mild and self- limiting. The undamaged skin or mucosal surfaces are the primary barriers to infection. Desiccation تيبس وتقشر الجلد, epithelial cell turnover, fatty acids, and the low PH of the skin are believed to be

important factors in host resistance . in addition , the bacterial flora of the skin and mucous membranes compete with fungi and delay their unrestricted growth . alterations in the balance of the normal flora by use of antibiotics or changes in nutrition allow fungi such as *C. albicans* to proliferate , thus increasing the likelihood of entry and infection . destruction of the natural barriers by trauma or foreign bodies allows entry of fungi into sterile areas of the body . as in all infections, the outcome is determined by the virulence of the infecting organism, the size of the inoculum and the competence of the host defenses.



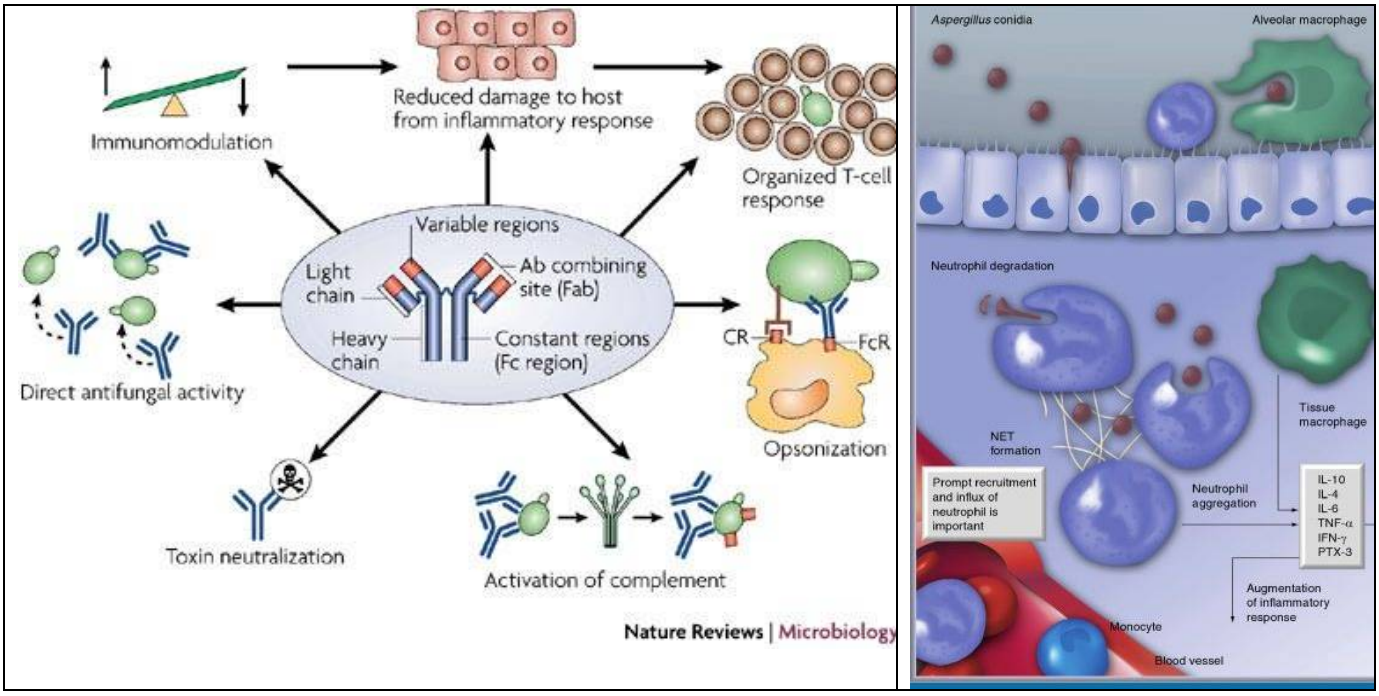
Spread and multiplication

Within host tissue , fungi are controlled by a variety of nonspecific mechanisms . using *C. albicans* as an example , the fungistatic effect of serum has been shown to be suitable, in part of it to transferrins (the human iron- binding proteins that remove iron from microbes they need for making respiratory enzymes) , In addition, β-globulins found in serum cause a non immunological clumping of *Candida* and facilitate their elimination by inflammatory cells .tissue reaction to the presence of fungi varies with the species, the site of proliferation, and the duration of infection. Some mycoses are characterized by a low rating inflammatory response, which does not eliminate the fungi .phagocytosis by neutrophils is the primary mechanism that prevents the establishment of fungal infections and usually its effective such as with *Candida* and *Aspergillus* infections but fungal cells can sometimes persist within macrophages or giant cells without being killed and spread to other organs of the body within those cells such as *H. capsulatum* and *C. neoformans* .

In general, nonspecific inflammatory reactions are critically important in eliminating fungi.

Fungi that are too big to be ingested can be killed by immunological mechanisms depending on the fact that Fungal cells and their extracellular products are highly antigenic and inducing both cellular and humoral responses .evidence found that

antibodies play a role in the elimination of fungi from the body. along with complement ,antibodies play a part in the extracellular killing of *A.fumigatus* and pseudohyphae of *C.albicans* by lymphocytes and phagocytic cells . Instead of (ingesting)"and (digesting)the fungi , phagocytic cells appear to secrete lethal lysosomal enzymes. Resistance to fungal disease is due mainly to cellular or T-lymphocyte-mediated immunity .This can be in expose from animal experiments and from the clinical observation , that patients with depressed cellular immunity are especially prone to invasive and serious systemic fungal disease .For example ,patient with AIDS commonly have mucocutaneous Candidiasis and serious systemic infections with *C.neoformans*.



Damage

Fungi that cause invasive disease are not known to secrete toxins that harm the host . tissue damage most probably results from direct invasion with disruption, destruction of vital structures, and toxic effects of the inflammatory response. Fungi may also grow as masses of cells (fungus balls) that can blocked bronchi in the lung or tubules or even ureters in kidneys, leading to obstruction of outflow of biological fluids (sputum,urine) and secondary infection and tissue damage , such as with Aspergillosis .

☆Immunity to Fungal Disease:

There are two type of immunity to fungal infection : **Nonspecific immunity &Specific immunity** but we can discussed it in another way as the following :

1/Inflammatory response –macrophages, PMNs are most effective killers effective for most fungi Drawn to site of fungal infection by chemotactic factors produced by fungus , also Fungi can stimulate IL-1 and TNF-α to Enhances infiltration of PMNs , PMNS kill by Oxygen dependent and independent mechanisms .

2/Humoral Immunity
• Many antigenic components – led to antibodies formed
• *But* ineffective for most

3/Cell-mediated Immunity
• Most important for fungal infections

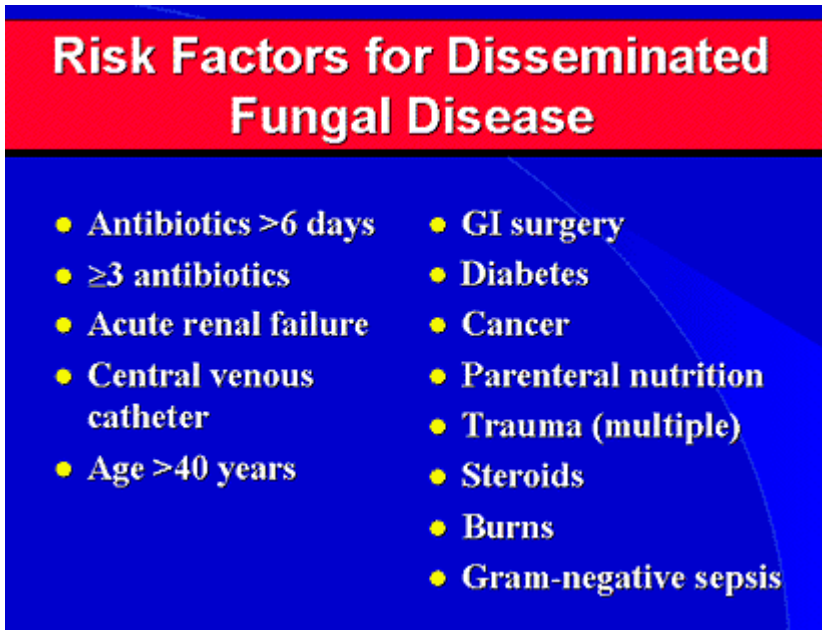
Epidemiology

• **Endogenous** – intestinal, vaginal flora
• **Exogenous** –
– Infectious molds (conidia) mature in the environment
– Conidia inhaled
• **Transmission** – human to human only with dermatophytes (ringworm)
• Some are geographically restricted

Most human fungal infections are caused by fungi that grow as saprophytes in the environment. Infection is acquired by inhalation, ingestion or traumatic implantation. Some yeasts are human commensals and cause endogenous infections when there is some imbalance in the host. Many fungal diseases have aworldwide distribution, but some are endemic to specific geographical regions, usually because the causal agents are saprophytes restricted in their distribution by soil and climatic conditions.

Incidence

The incidence of all the mycoses is related to factors that affect the degree of exposure to the causal fungi, such as living condition , occupation and spare time activities.such as risk factors for Disseminated fungal disease in the following table:



Dermatophytosis of foot(athletes foot) with associated infections of nails and groin, occurs most commonly in swimmers, sportspersons and industrial workers who use public bathing facilities.
Animal dermatophytosis is an occupational hazard for farmers, veterinarians and others closely associated with animals.
Agricultural workers in warm climates who wear little protective clothing frequently take subcutaneous infections following minor injuries from dealing with plants.
In many developing countries the acquired immune deficiency syndrome(AIDS) pandemic has been associated with a marked increase in the rate of opportunistic fungal infections. High mortality rates have been reported from countries where sufficient treatment is often unavailable.
increased international travel and tourism has also led to a rise in the number of cases of disease among individuals who normally reside in countries far from the endemic areas.

Diagnosis

Diagnosis of fungal infections is based on a combination of clinical observation and laboratory investigation.

①Clinical investigation

Superficial and subcutaneous mycoses often produce characteristic lesions, but they may also closely look like and be confused with other diseases. In addition the appearance of lesions may be modified beyond recognition by earlier therapy for example with topical steroids.
The first indication that a patient may have a systemic mycosis is often their failure to respond to antibacterial antibiotics. As early diagnosis significantly increases the chances of successful treatment , it is important that the possibility of fungal involvement be considered from the beginning, particularly in those known to be at risk of developing a fungal infection . computed tomography is widely used to help diagnose *Aspergillus* infections and other invasive mycoses.

Laboratory diagnosis

Laboratory diagnosis depends on:

- Recognition of the pathogen in tissue by microscopy
- Isolation of the causal fungus in culture
- The use of serological tests
- Detection of fungal DNA by the polymerase chain reaction (PCR)

But at the first , It is important that the correct type of specimen, together with enough clinical data, is sent to the laboratory so that the appropriate investigations can be carried out . information on factors such as travel , animal contacts and the occupation of the

patient enable the Laboratory staff to direct their investigations towards aparticular fungus or group of fungi when appropriate.

Fungal Disease	Specimen Types
SUPERFICIAL MYCOSES	Skin and Nail Scrapings
CUTANEOUS MYCOSES	Skin, Hair and Nail Scrapings Mucous membranes Ear, Eye
SUBCUTANEOUS MYCOSES	skin scrapings pus tissue Biopsy
Dimorphic Systemic mycoses	Blood and Bone Marrow Cerebrospinal Fluid (CSF) Sputum& Bronchial Washings Tissue Biopsies Urine Serum
Opportunistic Systemic mycoses	Blood Cerebrospinal Fluid (CSF) Sputum& Bronchial Washings Tissue Biopsies Serum

Direct microscopy

Most specimens can be examined acceptably in **wet mounts** after partial digestion of the tissue with **10-20% KOH** .addition of **calcofluor white** and following examination by fluorescence microscopy enhances the detection of most fungi, as the **fluorescent hydroxide- calcofluor** binds to the fungal cell walls. **Gram films** may also be used for the diagnosis of yeast infections of mucous membranes. **Giemsa staining** of smears is also used for detection of the yeast cells of *H. capsulatum* because of their small size.

Histology

Invasive procedures are required to obtain specimens for histological examination . although sometimes necessary to provide evidence of invasive disease, such procedures are often impracticable on patients who are already seriously ill . **haematoxylin** and **eosin** staining is not often of value for demonstrating fungi in tissue and specific fungal stain such as **periodic acid – Schiff** and **Grocott- gomorimethenamine- silver** are widely used .

Culture

Most pathogenic fungi are easy to grow in culture. **Sabouraud dextrose agar** and **4% malt extract agar** are most commonly used . these may be supplemented with **chloramphenicol** (50mg/l) to minimize bacterial contamination and **cycloheximide** (500mg/l) to reduce contamination with saprophytic fungi. Many fungal pathogens have an optimum growth temperature below 37 C . so cultures are incubated at 25-30 C and at 37C , with some dimorphic pathogens - enriched media such as **brain – heart infusion** or **blood agar** are used to promote growth of the yeast phase .

Many fungi develop relatively slowly and cultures should be retained for at least 2-3 weeks up to 6 weeks in some cases before being discarded ; yeast usually grow within 1-5 days. Moulds are identified by their macroscopic and microscopic morphology. Yeast are identified by sugar fermentation and their ability to assimilate carbon and nitrogen sources.commercial kits are available for the identification of medically important yeasts.

Culture may provide clear evidence of fungal infection when established pathogens are isolated or when fungi are recovered from normally sterile sites. However, when commensals such as *Candida* species are isolated, results must be interpreted according to the quantity of the fungus isolated, the source and clinical evidence.

Serology

The most common tests for fungal antibodies are :

- Immunodiffusion
- Countercurrent immuno-electrophoresis
- whole cell agglutination
- complement fixation
- enzyme-linked immunosorbent assay (ELISA)

for antigen detection the following are used:

- latex particle agglutination
- ELISA

Polymerase chain reaction

detect ion of fungal DNA in clinical material, principally blood, serum, broncho-alveolar lavage fluid and sputum is increasingly used for diagnosis.

Treatment

An **antifungal medication** is a medication used to treat fungal infections topically as an component in creams, ointments, lotions, soaps, and shampoos. In addition to USING IT

such as athlete's foot, ringworm, candidiasis (thrush), serious systemic infections such as cryptococcal meningitis, and others.

There are relatively few therapeutically useful antifungal agents compared with the large number of antibacterial agents that are available. As fungi and human beings are both eukaryotes, most substances that kill or inhibit fungal pathogens are also toxic to the host. Antifungal agents vary significantly in their spectrum of activity. Most utilize differences in the sterol composition of the fungal and mammalian cell membranes, although the echinocandins (caspofungin&micafungin)interfere with β-glucan synthesis in the fungal cell wall.

Most antifungal agents are available only for tropical , relatively few can be administered systemically.

Amphotericin b and the echinocandins are given parenterally because of poor absorption from the gastro- intestinal tract.

Fluconazole, itraconazole, voriconazole and flucytosine are available for oral or parenteral administration.Terbinafine and griseofulvin are usually administered orally.

Amphotericin b has long been the treatment of choice in life-threatening disease, despite its toxicity, liposomal and lipid complex formulations are less toxic but much more expensive.

Antifungal prophylaxis is often used to help prevent opportunistic infection in patients undergoing solid- organ, blood or marrow transplants and in those with haematological malignancies. Oral or topical antifungals are also used to prevent recurrent vaginal candidosis.

Primary or acquired resistance is not a major problem. Resistance to azole antifungals is sometimes encountered, especially after prolonged fluconazole therapy with oropharyngeal candida infections in persons with AIDS.

Mechanism of action

Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host. Unlike bacteria, both fungi and humans are eukaryotes. Thus fungal and human cells are similar at the molecular level. This makes it more difficult to find or design drugs that target fungi without

affecting human cells. As a consequence, many antifungal drugs cause side-effects. Some of these side-effects can be life-threatening if the drugs are not used properly.

Adverse effects

Apart from side-effects like liver-damage or affecting estrogen levels, many anti-fungal medicines can cause allergic reactions in people. For example, the azole group of drugs is known to have caused anaphylaxis.

There are also many drug interactions. Patients must read in detail the enclosed data sheet of the medicine. For example, the azole antifungals such as ketoconazole or itraconazole can be both substrates and inhibitors of the P-glycoprotein, which (among other functions) excretes toxins and drugs into the intestines. Azole antifungals also are both substrates and inhibitors of the cytochrome P450 family CYP3A4, causing increased concentration when administering, for example, calcium channel blockers, immunosuppressants, chemotherapeutic drugs, benzodiazepines, tricyclic antidepressants, macrolides and SSRIs.

Classes

Antifungal drugs can be grouped into classes or groups based on their site of action as following:

***1. Polyene Antifungal Drugs -**interact with sterols in the cell membrane (ergosterol in fungi where it is important for membrane fluidity and integrity. It has a "sparking" function in which small amounts are necessary for the cell to complete the cell cycle, grow, and divide., In contrast, there is cholesterol in humans) to form channels through which small molecules escape. Examples: Amphotericin, nystatin, and pimaricin .

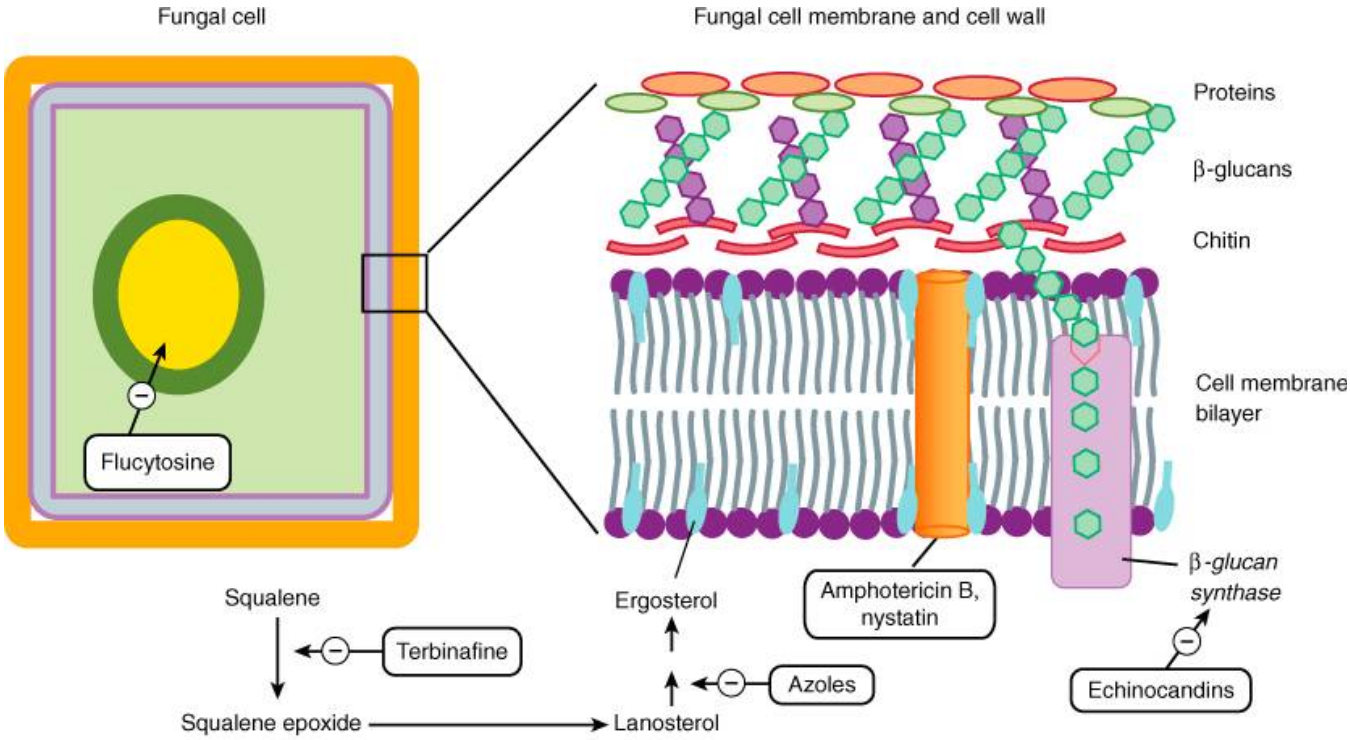
***2. Azole Antifungal Drugs**-inhibit cytochrome P450-dependent enzymes (particularly C14-demethylase) involved in the biosynthesis of ergosterol. Examples: Fluconazole, itraconazole, and ketoconazole .

***3. Allylamine and Morpholine Antifungal Drugs –Allylamines** inhibit ergosterol biosynthesis at the level of squalene epoxidase. Examples: Allylamines (naftifine, terbinafine) The morpholine drug, amorolfine, inhibits the same pathway at a later step.

4. Antimetabolite Antifungal Drug -5-Fluorocytosine acts as an inhibitor of both DNA and RNA synthesis via the intracytoplasmic conversion of 5-fluorocytosine to 5-fluorouracil.

5. **Echinocandins** –inhibit cell wall biosynthesis.

6. Other –Griseovolvin and many topical compounds (tolnaftate, undecylate) inhibit fungal growth but are too toxic or otherwise inappropriate for systemic infections.for example : Griseofulvin Acts as a fungistatic agent by binding to microtubules causing inhibition of mitosis.



Source: Katzung BG, Masters SB, Trevor AJ: *Basic & Clinical Pharmacology, 11th Edition:* http://www.accessmedicine.com

شكل: يوضح مواقع عمل بعض المضادات الفطرية المستخدمة في الوقت الحاضرلعلاج عدد من الاصابات الفطرية الشائعة .

Antifungal drug resistance

The limitation to the similarity between antifungal and antibacterial resistance mechanisms is that some general classes of resistance mechanisms have not yet been identified in fungi. Resistance to antibacterial agents results from modification of the antibiotic, modification of the antimicrobial target, reduced entrance to the target, or some combination of these mechanisms. Antibiotic modification may be the most important mechanism of resistance to the β-lactam (β-lactamases) and aminoglycoside (aminoglycoside-modifying enzymes) classes of antibacterials. In contrast, there are almost no data to suggest that antifungal modification is an important mechanism of antifungal resistance. On the other hand, **accumulating evidence suggests that both**

target alterations and reduced entrance to targets (sometimes in combination) are important mechanisms of resistance to antifungal agents, such as :

- Alterations in membrane sterols
- Enzyme mutations – 5FC permease
- Efflux pumps exclude drug from cytoplasm

Antifungal Susceptibility Testing

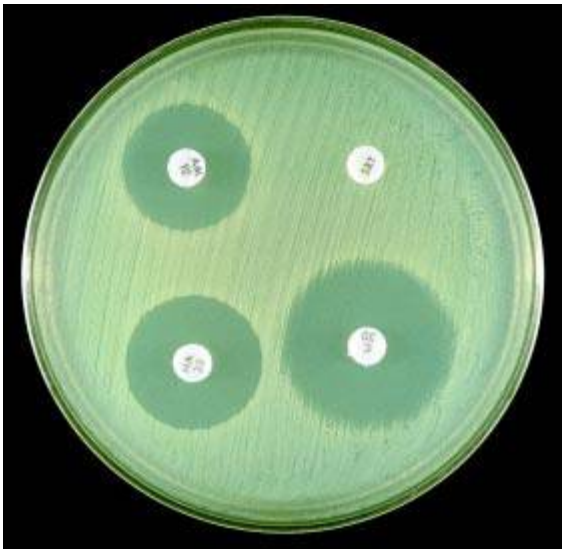
It is now possible for the clinical microbiology laboratory to make *in vitro* antifungal susceptibility tests on a wide range of yeasts and moulds. The aim of this lab is to a provide review of what is currently available to the clinical laboratory along with some practical explanation on antifungal susceptibility testing for using it in the treatment, These susceptibility tests include the introduction of standard reference methods for both yeasts and moulds by the CLSI (The Clinical and Laboratory Standards Institute) .

ANTIMICROBIAL SUSCEPTIBILITY TESTING METHODS ARE DIVIDED INTO TYPES BASED ON THE PRINCIPLE APPLIED IN EACH SYSTEM. THEY INCLUDE:

DIFFUSION	DILUTION	DIFFUSION&DILUTION
Stokes method	Minimum Inhibitory Concentration	E-Test method
Kirby-Bauer method	i) Broth dilution	
	ii)Agar Dilution	

① Kirby – Bauer method (Disk – diffusion method)

Its a most common method using in the clinical laboratory with yeast but rarely with moulds for tested antifungal agent because The sizes of the zones of inhibition should be taken after 24hr not longer that to prevent losing the activity of antifungal agent and this may led to recurrent growth of sensitive microorganisms .

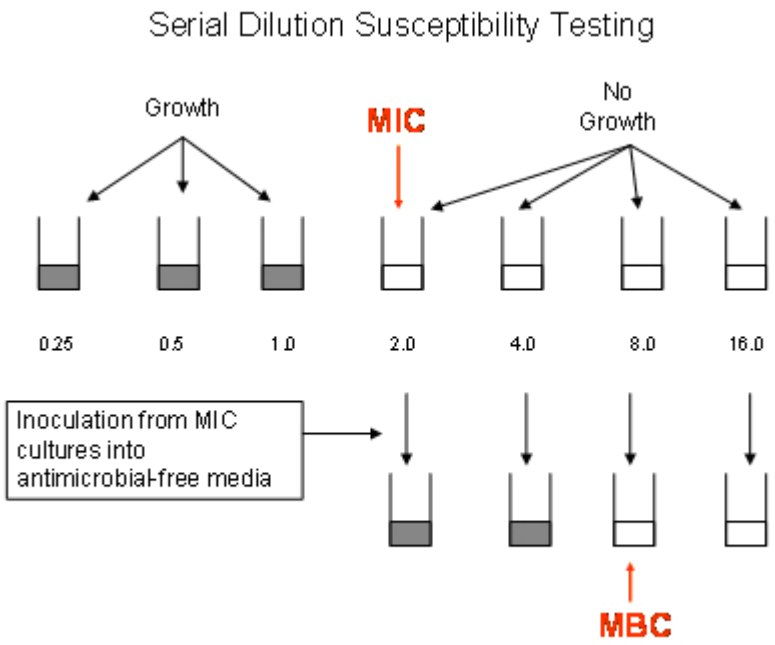


②Dilution Methods

Dilution susceptibility testing methods are used to determine the **minimal concentration of antifungal to inhibit or kill the microorganism (MIC&MFC)**. This can be achieved by dilution of antimicrobial in either agar or broth media. Antimicrobials are tested in $\log_2$ serial dilutions (two fold).

=Minimum inhibitory concentration (MIC) هو اقل تركيز من المضاد الحيوي يقلل عدد المستعمرات النامية بمقدار ٩٩.٩% من المزروع الاصلي

=Minimum fungicidal concentration (MFC) اقل تركيز يؤدي الى قتل الفطر



③ طريقة Etest

This is an agar diffusion method using a strip with a predefined concentration gradient of the antimicrobial agent being tested. This gradient allows for an MIC determination to be made. Etests have been extensively used for susceptibility testing of bacteria and they are also available for antifungal agents, including Amphotericin B, Ketoconazole , Itraconazole, Fluconazole, Voriconazole and 5-Fluorocytosine. The two difficulties with Etests against fungi have always been which medium to use and with the reading of the end point. The later problem remains but it is now recommended that modified RPMI-1640 agar supplemented with 0.2% glucose be used .

Etest

	
E Test. Test for sensitivity of *Aspergillus* to Itraconazole at different concentrations. In this case the *Aspergillus* was resistant to Itraconazole.	E Test for *Aspergillus* sensitivity to Voriconazole. In this example *Aspergillus* was sensitive to Voriconazole.

Clinical or medical virology is a branch of medicine , which deal with isolating and characterizing viruses responsible for some human diseases by various direct or indirect techniques (cellular Cultures, serologist, biochemistry, molecular biology). It also deal with proving the antiviral therapeutics efficiency in treatment.
As we know …Study of viruses is given us understanding for many host cellular mechanisms and steps of viral replication cycle to allowed designer for synthetic new and active antiviral drugs (such as for the influenza A virus).

Viral pathogenesis
Viral pathogenesis can be described as the process by which the virus interacts with its host to produce disease.

Pathogenic mechanisms of viral disease include :
(1) attaching of virus at the sites of entry
(2) local replication at the site of entry
(3) spread within this site or moving to target organs (disease sites)
(4) spread to sites of shedding of virus into the environment.

Therefore in this is process (which involves virus–host interaction) both viral and host factors have effects on the pathogenesis of viral disease.as following :

1/ Viral factors
Tropism(viral affinity)& Viral virulence
Tropism It means The ability of viruses to infect only certain cell types because of the presence of specific viral receptors on the cell surface that bind to viral surface proteins.

While Viral virulence is defined as the amount of virus required to produce disease or death in 50% of of experimentally infected animals.

Spread(transmission)

The mechanism of viral spread has a significant role in viral pathogenesis. That can be divided to :

Horizontal transmission	Vertical transmission
• Direct contact eg.,warts virus • Aerosols eg., influenza virus • Fecal- oral route eg.,polio virus • Sexual eg., HIV • Organs transplantation eg.,CMV • Infected blood eg., HIV • Ocular eg.,Adenovirus • Vectors(non-living& living) eg.,Yellow fever • Zoonotic transmission eg.,Rabies	• From mother to fetus eg., hepatitis B virus

Viral persistence

Many viruses cause persistent infection, which can be **latent** as in herpes virus infection, or **chronic** as in hepatitis B virus infection. In latency the virus lies dormant. The mechanisms of latency are not understood very well but the virus reactivates from time to time to cause localized infection, as in the case of herpes simplex virus, In chronic infection the virus replicates and continues to cause damage but in low rate comparing to acute infection. the best strategy that enables Viruses to persist and cause latent or chronic infection are :

1/ immune modulation. Many viruses that cause persistent infection avoid the specific and nonspecific immune defenses in several ways such as :

1.Limitation of recognition molecules on infected cells. That can be done by :

a.Restricted expression of viral antigens by cellular and viral factors such as a result of DNA methylation or lack of appropriate transcription factors in host cells or viral DNA replication and transcription are maintained at very low levels.e.g., HIV, measles virus .
b. Avoidance of neutralizing with antibodies by spreading directly from cell to cell.e.g., measles virus.
c.Viral antigenic variation e.g., HIV.
d.Blocking antibody for prevents the binding with the neutralizing antibody e.g., measles virus.
e.Decreased or altered expression and intracellular distribution of antigen-presenting molecules such as cell major histocompatibility complex MHC class I that recognition strange molecules e.g., CMV, adenoviruses.
f.Restricted expression of the cell adhesion molecules e.g., EBV, CMV.
g.Budding of virus particles into cytoplasmic vacuoles, resulting in masked virus production.

2.Altered and Inhibition of lymphocyte and macrophage functions, including modified production of cytokines and immunosuppression e.g., HIV-1, HIV-2, EBV.

3.Infection and multiplication in immunologically private sites e.g., HSV-1, HSV-2, VZV in the central nervous system.

4. Inhibition of immune and nonspecific defenses e.g., inhibition of complement-mediated lysis due to an increased production of inhibitory factors (e.g., CD55 for inhibition of interferon),.

5.Immune tolerance .eg., Immune tolerance to the surface protein of HBV appears to be one of the factors involved in the development of the carrier state.

6.Mobility of latently infected cells within the host.

2/ modulation of viral gene expression. Examples include down regulation of some viral genes by viral or cellular regulatory gene products (e.g., HIV, HPVs), specific latency-associated proteins (e.g., HSV-1, HSV-2) and viral variants (e.g., HIV, measles).

2/ Host factors

Host response

Disease appearances may be the direct result of infection or may be immune mediated as a result of the host immune response to the infection eg., Hepatocellular damage in HBV infection is a result of destruction of infected hepatocytes by the cytotoxic T-cells. In influenza, most of the symptoms are mediated by interferon produced in response to the infection. Human immunodeficiency virus induces immunodeficiency by destroying the helper T-cells (CD4) of the cell-mediated immune system.

Virus Diagnostic techniques

Detection of viruses in a patient's secretions or tissue provides direct evidence of recent or persist infection .This can be done by:

- **detection of viral antigens or viral antibody by serology.**

Serology is used widely to diagnose viral infections as many of the viruses cannot be easily cultured. Here this word 'Serology' means the using of serum to detect both antibody and antigen involved in viral infection (e.g. hepatitis B surface antigen),for this purpose Several techniques have been developed- but the essential principles are similar for all.

Antibodies are produced as a host response to viral infection eg., **Ig A** is produced at the local site of infection and provides local immunity as in gut or respiratory tract. The generalized humoral immune response is mounted by B lymphocytes and the first antibody to appear is the **IgM** which can be detected in early days after an acute infection. after that , Some of the B lymphocyte clones then switch over to producing

IgG antibody, which appears usually from(7–15) days after onset of infection. Both classes of antibodies continue to rise in response to the infection, peaking at about 6 weeks post infection. Viral specific IgM then declines and is normally undetectable by about 3 months after infection. but IgG antibody persists for life and is responsible for providing lifelong immunity to the particular virus. Therefore , Past infection or immunity is diagnosed by: demonstration of virus specific IgG alone (and absence of IgM).

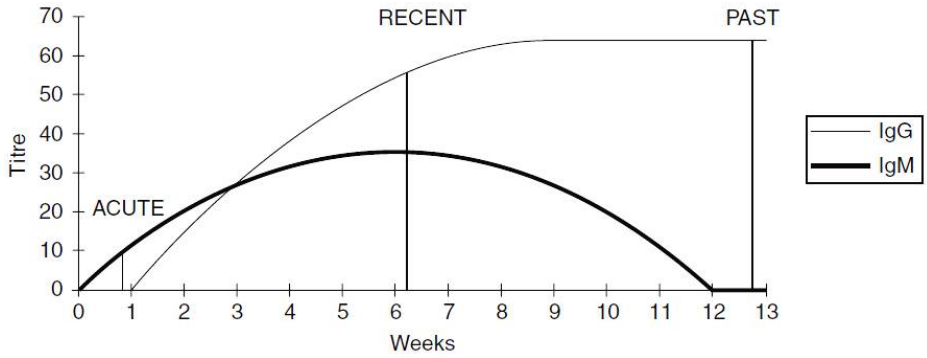


Fig. 47.1. Figurative representation of serological response to viral infection.

Table 47.1. *Diagnostic uses of the serological techniques.*

Test	Example of use
Complement fixation test (CFT)	Respiratory viruses – measures total antibody, is quantitative; to diagnose recent infection acute to show rise in titre.
Enzyme-linked immunosorbent assays (ELISA)	IgG/IgM antibody – rubella, measles, mumps, HIV, hepatitis A etc. Antigen – hepatitis B surface antigen in serum samples, norovirus and rotavirus antigen in faeces.
Immunofluorescence (IF)	IgG/IgM antibody – EBV, VZV. Antigen – RSV, influenza and other respiratory viruses in respiratory secretions.
Latex and gel particle agglutination	Antibody – rubella, toxoplasma. Antigen – rotavirus, norovirus.
IgG avidity assays	To confirm recent CMV, rubella and toxoplasma infections.

- **virus culture (cell or tissue culture)**

viruses are fastidious intracellular organisms and therefore living cells are required to grow viruses in the laboratory. So,Viruses, like bacteria can be cultured in the laboratory. Therefore , Many cell lines have been developed to support the growth of different viruses. but A single type of cell line is not sufficient for all viruses, as specific viruses need specific receptors on the cell surface to attach for entry into the cell and to initiate replication. The presence of specific cell receptors on the cell surface determines which

viruses will be able to infect them, and this is called 'viral cell tropism'. For this reason many cell lines have to be maintained in a diagnostic laboratory. Another problem in the laboratory is to maintain these living cells in culture long enough to allow sufficient virus growth,therefor A suspension of cells in growth medium (consists of a buffer plus calf serum to provide protein and amino acids, and antibiotics to prevent bacterial overgrowth) used and The patient's specimen is then added and cells incubated at 37 C to allow the virus to grow (usually 33C for respiratory viruses). The tubes are examined daily to look for evidence of virus growth(take from a day to weeks), If virus is present then it kills off the cells; depending upon the cell line and the virus growing in it this gives a typical appearance in the cell sheet and is referred to as cytopathic effect (CPE)to see if the suspected virus is present in it.
Cell culture, although a very sensitive and specific technique, and requires considerable technical expertise. Results may take up to a few weeks and the specimens need to be transported quickly and under correct conditions to the laboratory to maintain the viability of the infecting virus. Many viruses, such as the hepatitis viruses, papilloma viruses, parvovirus B19, rotavirus and norovirus, cannot be grown in cell culture and others, such as EBV and HIV, need special cells and therefore are not suitable for culture in a routine diagnostic laboratory.

Cell lines could be taken from human or non-human sources and can be generally divided into the following:
_ **Continuous** – these are immortalized cell lines derived mostly from tumour cells or cells that have been transformed in the laboratory. Examples are HeLa (human cervical cancer cell line), hep2 and Graham 293 cells (transformed human epithelial cell line).
_ **Primary or semi-continuous** – as they can be maintained for only one or a limited number of growth cycles.They are more sensitive to infection by viruses, and fastidious viruses like VZV, CMV, and influenza virus will only grow in them. Examples areMRC5 (human lung fibroblast cell line), PMK (primary monkey kidney cell line).

- **electron microscopy – imagining of whole virus particles**

Viruses are below the resolution of light microscopy and therefore require an electron microscope for imagining. A limiting factor of electron microscopy (EM) is that =viruses belonging to the same family therefore they can not be distinguished from each other as they will have the same morphology (size, shape and surface characteristics). Therefore EM can not be used to make the differential diagnosis of a herpes simplex or chickenpox lesion, as both will contain a 'herpes' virus with exactly the same morphology . On the other hand EM is a useful tool in making a differential diagnosis of viral gastroenteritis, as rotavirus, norovirus and enteric adenoviruses all belong to a different family and can be distinguished from each other on the basis of their morphology.
Electron microscopy is expensive, technically demanding, requires specialist training and is relatively insensitive (requires a minimum of one million viral particles per ml or gram of specimen), and its use has therefore largely been limited to research institutions.

- **detection of viral genome (RNA or DNA) by molecular techniques.**

Although Molecular assays need a great deal of technical expertise and expensive equipment,The use of these techniques has been common in virology and replace other less sensitive methods of virus detection.that led to the discovery of new viruses, the study of viral resistance, designing of new antiviral drugs and vaccines because its sensitivity and specificity in addition to less cross-contamination problem.
Many are in use in routine diagnostic laboratories to aid viral diagnosis.
Those Molecular techniques that are in current and common use are described below:

DNA or RNA hybridization
A complementary RNA or DNA probe is used to bind to the DNA or RNA viral genome. The DNA–RNA hybrid can then be detected using a labeled monoclonal antibody. This technique can be used on tissue or fluid samples, in which in this case it called **in situ hybridization**, or the viral genome can first be transferred on to a blotting paper (dot blot hybridization).but To obtain a positive reaction, high numbers of copies of viral DNA or RNA are required; therefore the technique is relatively insensitive and has been used in specific cases.

Polymerase chain reaction (PCR)
It is a technique by which a single copy of DNA or RNA can be amplified more than a million times. But To detect RNA viruses, RNA has to be first transcribed to complementary DNA by means of an enzyme called reverse transcriptase; this type of PCR is referred to as **reverse transcription PCR** (**RT PCR**).

Many variations of the PCR technique are in use:

- **Multiplex PCR** – can detect several different viral genomes in a single reaction mixture. This allows detection of several viruses at the same time by a single test.
Useful for testing specimens from patients in whom a suspected infection may be caused by different unrelated viruses, e.g. respiratory viruses.
- **Real-time PCR** – here the amplification and detection steps of PCR occur at the same time. Therefore, the time taken to get a result is much shortened.
- **Quantitative PCR** – comparison of the amount of DNA or RNA present in the patient sample to a set of known positive standards included in the PCR assay is used to determine the virus quantity or viral load in the patient specimen.

Complete virus genomes of several viruses have been sequenced and identified. This allows typing and comparison of viral isolates for epidemiological purposes, and to establish the infection transmission chain. The most important clinical application, here are, for resistance testing to identify if the infecting virus may have any mutations that may confer resistance to the antiviral drug(s) in use.

Patient management(Prevention and treatment)

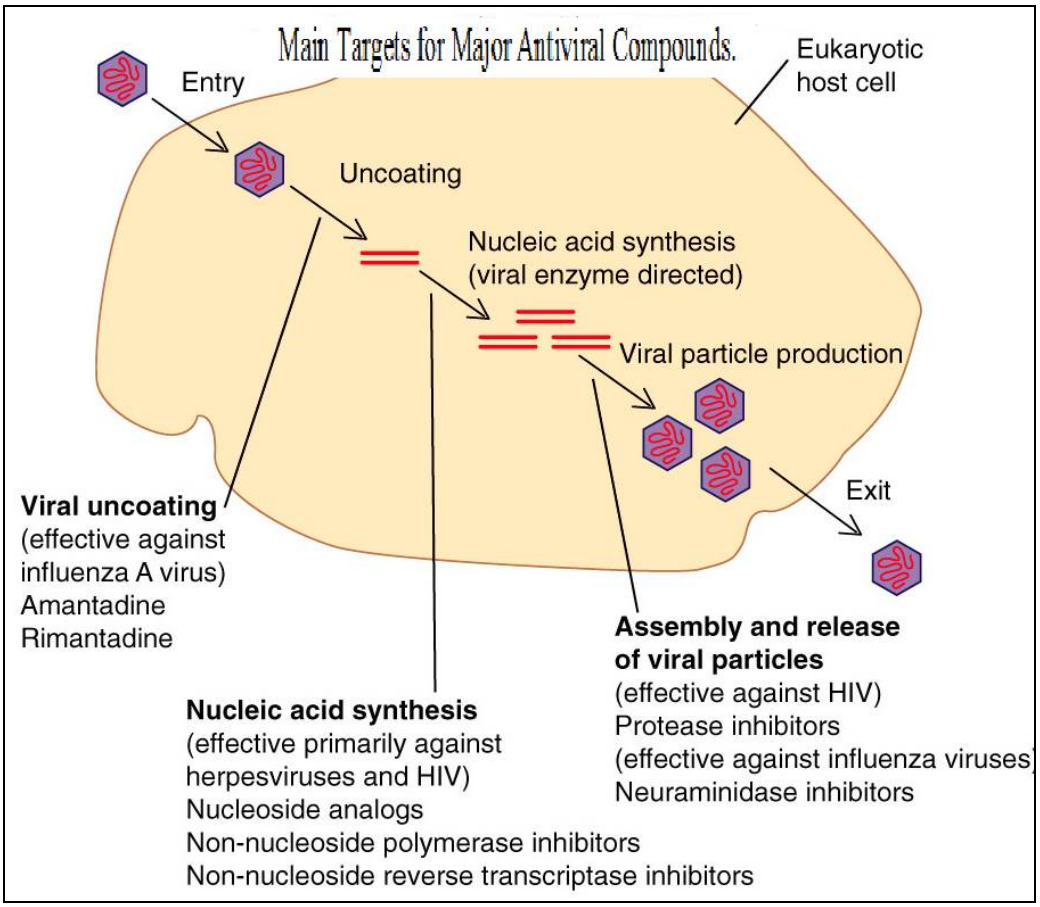
Because viruses use vital metabolic pathways within host cells to replicate, they are difficult to eliminate without using drugs that cause toxic effects to host cells in general. Therefore,The most effective medical approaches to manage viral diseases are vaccinations to Strengthening the immune response against viral infection , and antiviral drugs that selectively interfere with viral replication.

ANTIVIRAL DRUGS

Drugs that fight viral infections are called antiviral drugs. Antiviral drugs can work by:

- Interfering with the replication of viruses, Specific events in virus replication identified as main targets for antiviral agents are (viral adsorption, penetration, uncoating, and viral nucleic acid synthesis as well as viral protein synthesis).

- Strengthening the immune response against viral infection: These drugs include several types of interferons, immunoglobulins, and vaccines



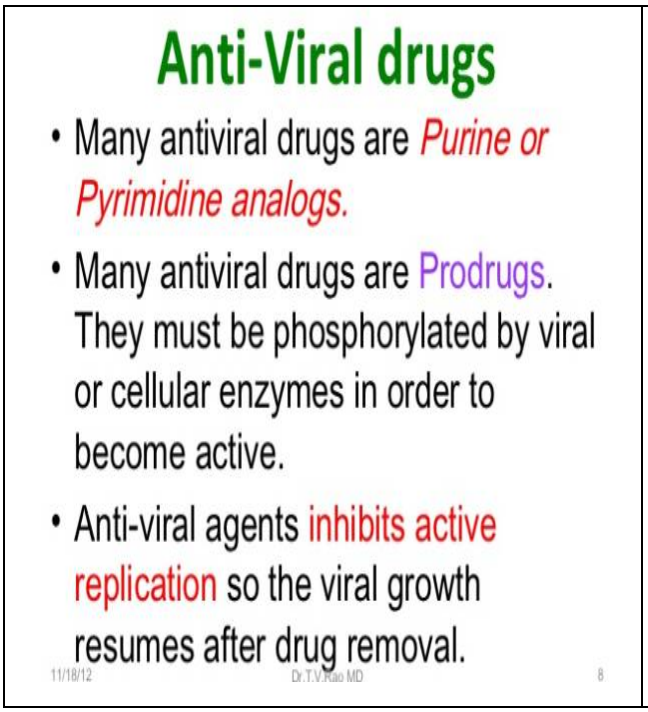
Because viruses are tiny and replicate inside cells using the cells' own metabolic functions, there are only a limited number of metabolic functions that antiviral drugs can target. In contrast, bacteria are relatively large organisms, commonly reproduce by themselves outside of cells, and have many metabolic functions that antibacterial drugs (antibiotics) can target. Therefore, antiviral drugs are much more difficult to develop than antibacterial drugs. Also, unlike antibiotics, which are usually effective against many different species of bacteria, most antiviral drugs are usually effective against only one (or a very few) viruses. Also we must know , Antibiotics are not effective against viral

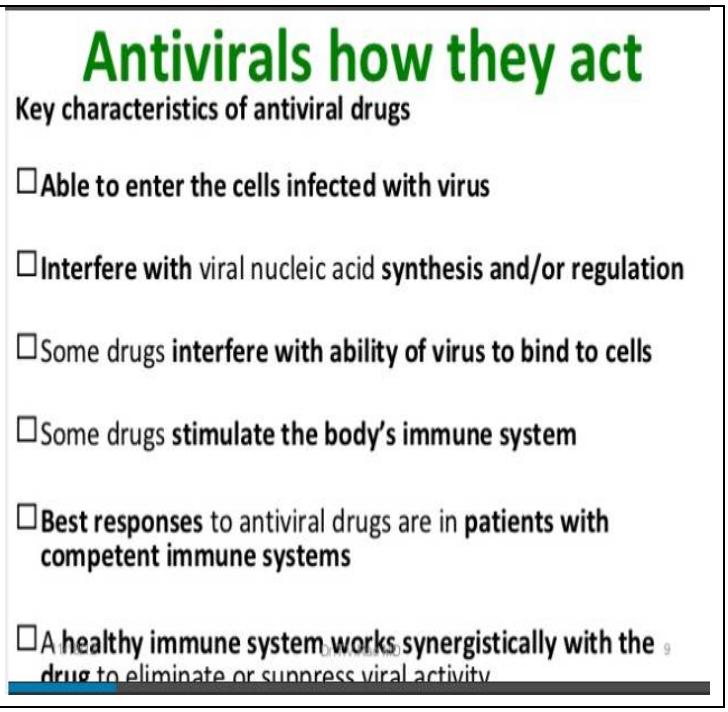
infections, but if a person has a bacterial infection in addition to a viral infection, an antibiotic is often necessary.

Most antiviral drugs can be given by mouth. Some can also be given by injection into a vein (intravenously) or muscle (intramuscularly). Some are applied as ointments, creams, or eye drops or are inhaled as a powder. but we must know , Antiviral drugs can be toxic to human cells. Also, viruses can develop resistance to antiviral drugs.

Antiviral drugs are often nucleoside analogues (false DNA building-units), which viruses mistakenly incorporate into their genomes during replication. The life-cycle of the virus is then stopped because the newly synthesized DNA is inactive. This is because these analogues lack the hydroxyl groups, which, along with phosphorus atoms, link together to form the strong "backbone" of the DNA molecule. This is called DNA chain termination. Examples of nucleoside analogues are **aciclovir** for Herpes simplex virus infections and **lamivudine** for HIV and Hepatitis B virus infections. Aciclovir is one of the oldest and most frequently suggested antiviral drugs. Other antiviral drugs in use target different stages of the viral life cycle. HIV is dependent on a proteolytic enzyme called the HIV-1 protease for it to become fully infectious. There is a large class of drugs called protease inhibitors that inactivate this enzyme.

So, Antiviral compounds can be divided into two groups:
(1) those that can interact directly with their target . e.g., amantadine, rimantadine, foscarnet and viral protease inhibitors.
(2) those that must first be activated intracellularly by phosphorylation to be at the active (generally triphosphate) forms. e.g., all nucleoside analogs such as acyclovir, ganciclovir, penciclovir or bromovinyldeoxyuridine……..etc.





Chemotherapeutic agent	Effect/indication
Adamantanamin (amantadine)	Inhibition of uncoating in influenza viruses
Acycloguanosine (acyclovir, Zovirax)	Inhibition of DNA synthesis in HSV and VZV
Dihydropropoxymethylguanosine (DHPG, ganciclovir, Cymevene)	Inhibition of DNA synthesis in CMV
Ribavirin	Inhibition of mRNA synthesis and capping. Infections with Lassa virus and perhaps in severe paramyxovirus and myxovirus infections
Nucleoside RT inhibitors (NRTI)	Inhibition of RT in HIV
Phosphonoformate (foscarnet)	Inhibition of DNA synthesis in herpesviruses, HIV, HBV
Protease inhibitors	Inhibition of viral maturation in HIV
Neuraminidase inhibitors	Inhibition of release of influenza viruses
Antisense RNA	Complementary to viral mRNA, which it blocks by means of hybridization (duplexing)

Viral VACCINATION

Vaccination is a cheap and effective way for preventing infections by viruses. Their use has resulted in a dramatic decline in morbidity (illness) and mortality (death) associated with viral infections such as polio, measles, mumps and rubella. Smallpox Vaccines can consist of live-attenuated or killed viruses, or viral proteins (antigens). Live vaccines contain weakened forms of the virus, which do not cause the disease but confer immunity, Such viruses are called attenuated. Live vaccines can be dangerous when given to people with a weak immunity (who are described as immunocompromised), because in these people, the weakened virus can cause the original disease. Biotechnology and genetic engineering techniques are used to produce subunit vaccines- where These vaccines use only the capsid proteins of the virus, Hepatitis B vaccine is an example of this type of vaccine. Subunit vaccines are safe for immunocompromised patients because they cannot cause the disease.
The yellow fever virus vaccine, a live-attenuated strain called 17D, is probably the safest and most effective vaccine ever generated.

Types of Vaccine

1. whole virus vaccines. either live or killed.
2. Subunit vaccines;- purified or recombinant viral antigen
3. Recombinant virus vaccines
4. Anti-idiotype antibodies
5. DNA vaccines

Live & Inactivated whole virus vaccines

Feature	Live vaccines	Dead vaccines
Dose con. & no.	low - Single	High- multiple
need for adjuvant	No	yes
Duration of immunity	many years	less
antibody response	IgG	IgA IgG
CMI	Good	poor
Reversion to virulence	possible	not possible
safety problems/ Disadvantages	1. Underattenuation or Mutation leading to reversion to virulence 2. Preparation instability 3. Contaminating viruses in cultured cells 4. Heat lability 5. Should not be given to immunocompromized or pregnant patients	1. Incomplete inactivation 2. Increased risk of allergic reactions due to large amounts of antigen involved

Synthetic Peptides

defined viral antigens or Synthetic Peptides Vaccines	
Advantages	**Disadvantages**
1. Production and quality control simpler 2. less toxic. 3. Safer in cases where viruses are establish a persistent infection 4. Possible even if virus cannot be cultivated	1. May be less immunogenic than inactivated whole-virus vaccines 2. Requires adjuvant 3. Requires primary course of injections followed by boosters 4. Fails to elicit CMI.

Anti-idiotype antibodies vaccines

The ability of anti-idiotype antibodies to stimulate antibodies formed (anti-anti-idiotype antibodies) that recognize a structure similar to specific part of the virus as antigen (epitope) that neutralize the virus as a foreign antigens. Anti-idiotypes may uses as viral vaccines, particularly when the antigen is difficult to grow or hazardous. They have been used to induce immunity against a wide range of viruses, including HBV, rabies, Newcastle disease virus and polioviruses.

=When one antibody binds to an idiotope of anotherantibody it is referred to as an anti-idiotypic antibody. The variable part of an antibody including the unique antigen binding site is known as the idiotype. The combination of epitopes within the idiotype (i.e. the idiotopes) is unique for each antibody

DNA vaccines

DNA vaccines	
Advantages	**Disadvantages**
• Plasmids are easily synthetic in large amounts • DNA is very stable • A DNA sequence can be changed easily in the laboratory. This means that we can respond to changes in the infectious agent • Mixtures of plasmids could be used that encode many protein fragments from a virus or viruses so that a broad spectrum vaccine could be produced • The plasmid does not replicate and encodes only the proteins of interest	• integration of plasmid into host genome leading to insertional mutagenesis • Induction of autoimmune responses (e.g. pathogenic anti-DNA antibodies) • Induction of immunologic tolerance (e.g. where the expression of the antigen in the host may lead to specific non-responsiveness to that antigen

Infection control

Infection control is a significant part of a clinical virologist's work. It is an important public health tool in the preventative and stop the spread of viral infections.

To do this we must first understand how viruses spread and entry to infect susceptible hosts cell . controlling virus infections should be done by :

- public education – for avoidance of infection, including vector control to protect from bites (mosquitoes, ticks, fleas etc.) for vector borne infections .
- screening programmes – for identifying infected patients to treat and to prevent transmission
- vaccination.

The aim of infection control in hospitals is to avoid spread of the nosocomial infection.

As we know , Certain groups of patients, such as the immunosuppressed, pregnant, neonates and elderly, are more susceptible to infected than others and may require special attention.

Outbreaks

Outbreaks of infection occur in both community and hospital settings. An outbreak is defined as the occurrence of two or more cases of the same infection associated in time

and place. Outbreaks may arise from a common source (eg., by food- or waterborne) or from person-to-person spread. For Outbreak investigation This requires founding the link between cases, preferably by molecular epidemiology to demonstrate either a common source or a person-to-person spread. So we must the :

Cluster : refers to a group of cases in a specific time and place.
Outbreak: An increase in the incidence of a disease than normally expected in localized area over a specific period of time.
Pseudo outbreak: Increase in detection of true cases that not related to true increase in cases by Change in local reporting practices,changes in diagnostic methods eg: misdiagnosis, laboratory or contamination.
Epidemic : The occurrence of illness in a defined region with an excess of normal expected frequency
Endemic: The constant presence of a disease within a given geographic area or population group.
Pandemic: An epidemic usually affecting a large proportion of the population, occurring over a wide geographic area such as a section of a nation, the entire nation, or a continent or the world.

Control of outbreaks
control an outbreak will depend upon the infecting agent, its source and suspected route of transmission. These consist of:

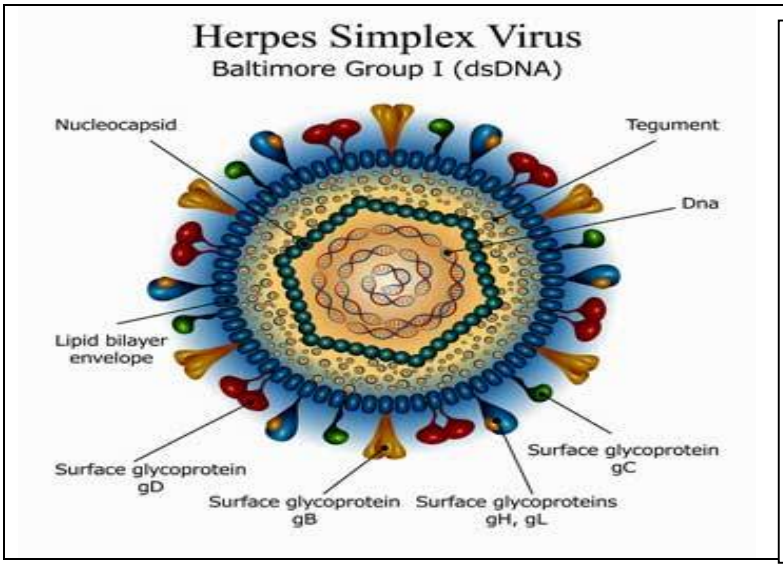
- _ Isolation of infected cases.
- _ Prophylaxis by vaccination, immunoglobulins or antiviral drugs as indicated.
- _ Investigation (and isolation) of new cases by: clinical signs of infection, laboratory screening tests for those with asymptomatic infection.

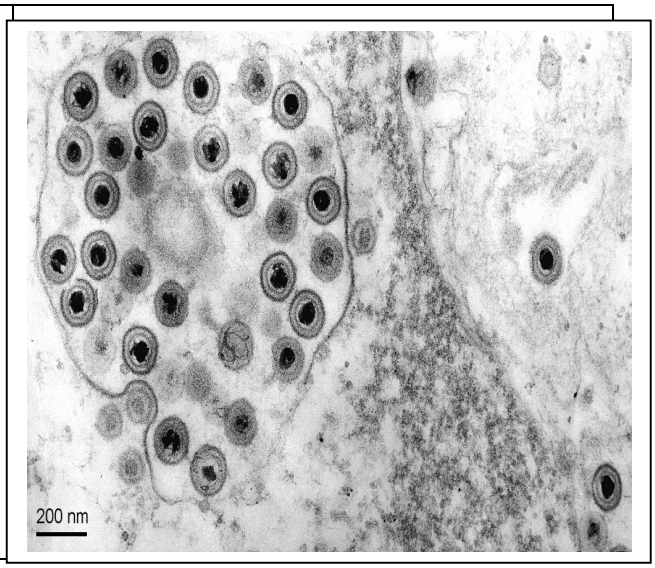
Herpesviruses

- Herpes Viruses are a cause of human viral diseases, second only to influenza and cold viruses.
- Are capable of causing hidden disease or remaining silent for many years only to be reactivated.
- Name *Herpes* comes from the Greek word *herpein* which means to creep.

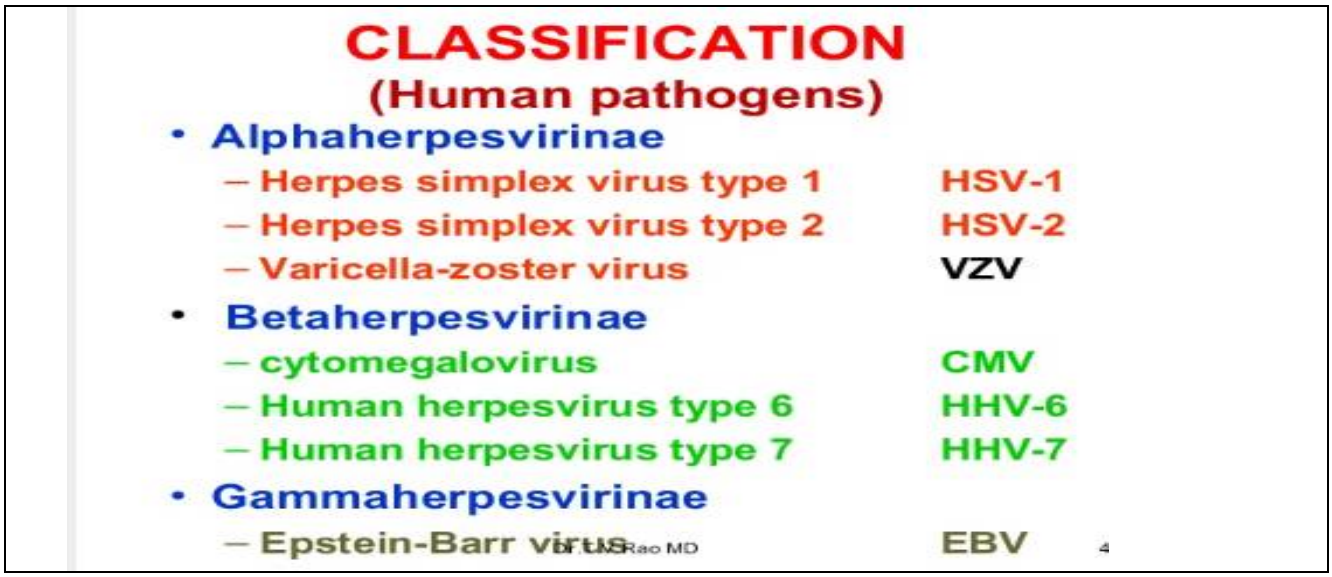
Properties of Herpesviruses

- Structure and Composition
  Spherical iscoahedron, (150-200) nm
  Double-stranded DNA, linear with large number of genes
  Enveloped
  Replication from nucleus (budding)





- Establish latent infections,Lifelong persistence.
- Significant cause of death in immunocompromised hosts(Systemic infections).
- Some can cause cancers
- Classification (human viruses):There are 25 families in the Herpeotoviridae but only some of them infect man .as following:



Replication of HSV

-Virus attachment and membrane fusion

- Viral host shutoff (VHS) protein released into the cytoplasm and initiates the degradation of host cell mRNA.
- α-Trans-inducing factor (αTIF) is transported to the nucleus.

-Capsid travels to nucleus where viral DNA is released, enters a nuclear pore and circularizes, where αTIF induces the expression of viral alpha genes

- The mRNAs for the alpha genes are translated on ribosomes
- The proteins then enter the nucleus and express the viral beta genes

The beta proteins are involved in degrading cellular chromatin and localizing cellular DNA to the inner side of the nuclear envelope (margination).

-Viral DNA is replicated as concatemers(is multiple copies of the virus DNA in one linear molecule called concatemer.) , Gamma proteins (structural) are expressed Capsid proteins self assemble and DNA concatemers are cleaved and packaged into capsids.

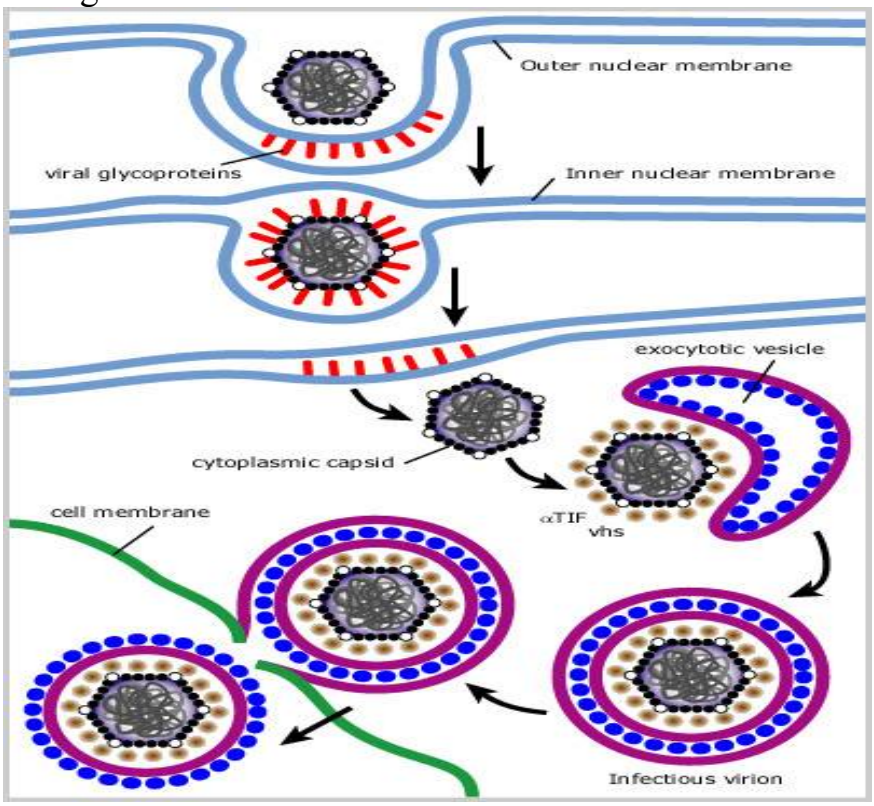
-Nuclear escape

Viral proteins induce budding of the capsid through both nuclear membranes

Thus, capsid escapes into the cytoplasm

-Viral proteins associate with the cellular vesicles…These proteins have affinity for the capsid proteins and cause the vesicle to wrap around the virus, providing it with an double-layered envelope

-Virus traverses the ER then Golgi prior to release from the cell….The outer membrane fuses with the plasma membrane and This permits the virus to leave the cell while retaining the inner membrane.



Epidemiology

- HSV-1 and 2 infections are life-long.
- The virus is found in the lesions on the skin but can be present in body fluids including saliva and vaginal secretions.
- As a result of poor hygiene in underdeveloped countries, HSV-1 antibodies are found in more than 90% of children.
- HSV-2 is normally spread sexually and is found in the anus, rectum and upper alimentary tract as well as the genital area.
- An infant can be infected at birth by a genitally-infected mother. Because of the infant's underdeveloped immune system, the resulting infection can be very severe and sometimes be deadly

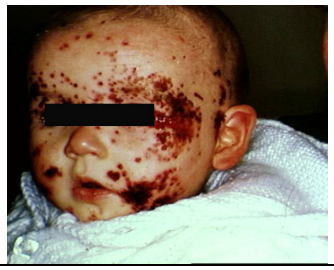
Herpesvirus Diseases

1/Herpes Simplex viruses

Two species :**HSV-1 & HSV-2** with Highly similar genomes.

HSV-1

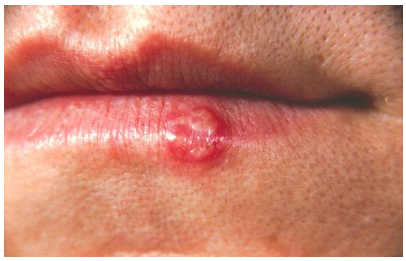
- **Eczema Herpeticum**
  - This is found in children with active eczema.
  - The virus can spread to other organs such as the liver and adrenals.



- **Oral Herpes - Cold sores**
  - often initially on the lips spreads to all parts of the mouth and pharynx.

-Symptoms: fever, vesicular and ulcerative lesions, edema, …..etc

Recurrence in some people throughout adult life



HSV-2

- Is usually causing genitalia (young adults).
- Primary infection is often asymptomatic but many painful lesions can be developed on the shaft of the penis and vulva, vagina, cervix and perianal region of women. In both sexes, the urethra can be involved.
- Genital Herpes infections can be accompanied by a variety of symptoms including fever, myalgia, glandular inflammation of the groin area (inguinal).
- Some patients have only infrequent recurrences but others experience recurrences as often as every 14-21 days.

Symptoms:Similar lesions and recurrence , Complications can occur,Transmission to newborn infant,Aseptic meningitis,Visceral herpes

Properties of Herpes Simplex Viruses
Type 1 and 2

- Similar in Organization
- Restriction Enzyme Differentiates
- H S V 1 contact with Saliva.
- H S V 2 Sexual
- Maternal infection (Genital Infection spreads to New Born)
- Replicates in 8-16 hours.

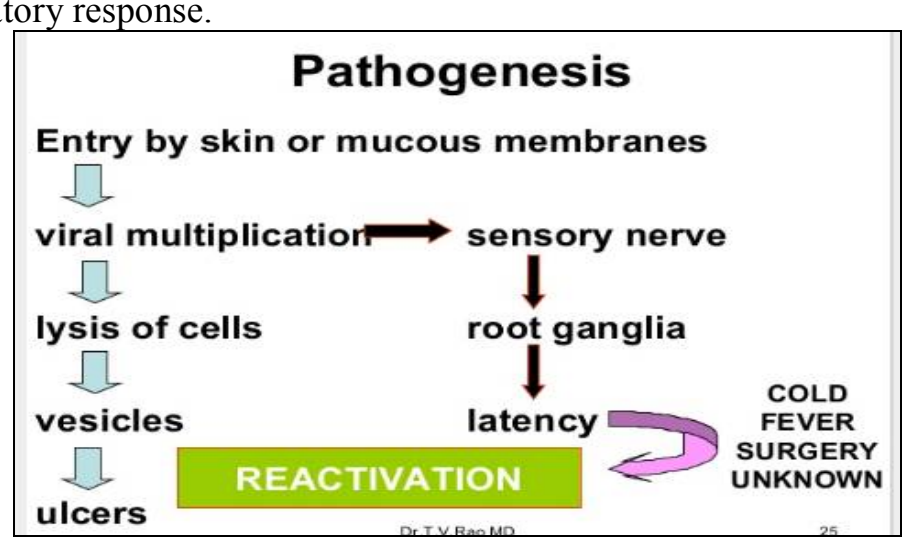
Virulence factors

binds complement C3b

is an Fc receptor for IgG

Pathology

Wide cellular tropism,Most common to dermal tissues (herpetic lesions) with Inflammatory response.



Transmission

Generally through direct contact with person shedding virus,Some people shed virus despite absence of lesions.Virus enters through mucosal tissues; cannot penetrate healthy skin.

Latent infections

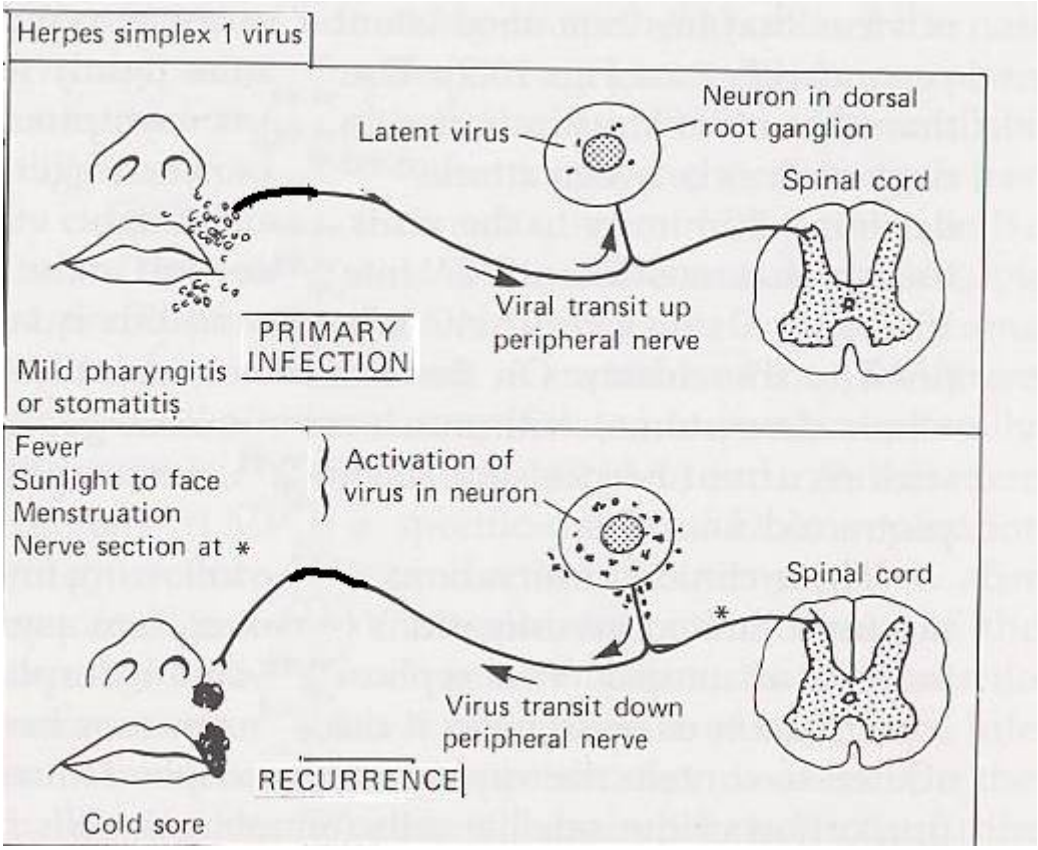
Virus sequesters in nerve tissues (immunoprivileged site)

HSV-1 in trigeminal ganglia

HSV-2 in sacral ganglia

Very few genes are expressed by infected cells

No immune response against infected cells



Immune response is not sterilizing

- Mothers Ig G protects for 6 months. •

-IgG, IgA, IgM--- Primarily Ig M Later Ig g produced. Main Participants in Immunity

- C M I and Killer Cells and Interferon play major role in immunity

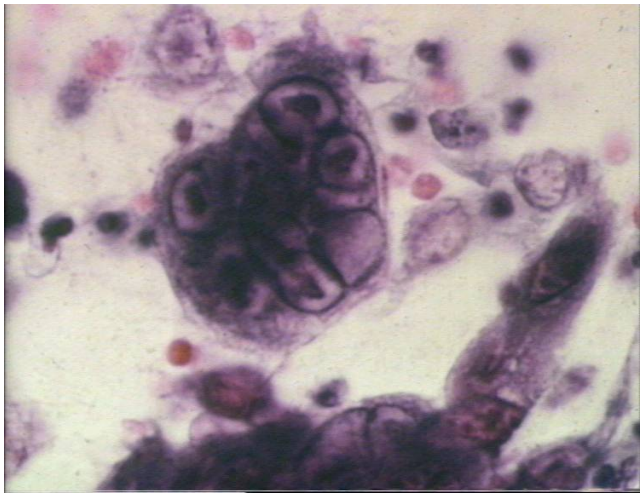
Viral proteins(α47 protein, ICP47 protein) block MHC class I pathway

$CD4^+$ T cells can kill infected cells

Laboratory Diagnosis

- Specimens for Diagnosis: •Saliva. •CSF •Vesicle fluid
- Cells may be obtained from the base of the lesion (called a **Tzank smear**) and histochemistry performed.

- Microscopy, (Electron Microscopy • Fluorescent Antibody)• Tzanck Smear - Intranuclear Type A Inclusion Bodies… These can be seen in the smears as multinucleated giant cells from skin scrapings and contain Cowdry type A inclusion bodies.



- Virus isolation:

- Chick embryo
- In Tissue Cultures Primary Embryonic Kidney Human

- Immunofluorescence
- Restriction digestion of viral DNA (HSV-1 vs. HSV-2)

-PCR- Used for systemic or encephalitic disease

- Serology- IgG appears in 4-7 days but Cannot discriminate HSV-1 from HSV-2

ELISA Test , Neutralization Tests , Complement Fixation Tests

Epidemiology

HSV-1	HSV-2
Most commonly acquired by children Most adults are seropositive	Most commonly acquired by young adults Sexually-transmitted disease Fetal/newborn transmission Increased risk for HIV infection

Treatment, prevention and control

Cheomtherapy : Acyclovir , Valacyclovir, Famciclovir

No vaccine is available