Extension Topic A: Infectious Diseases (Part 2)

1. Introduction

Pathogens have the ability to infect humans and cause diseases. This led to the development of vaccines to induce immunity against pathogens as well as antibiotics to treat bacterial infections.

Edward Jenner is considered the founder of vaccinology in the West in 1796, after he inoculated a 13 year old boy with vaccinia virus (cowpox), and demonstrated immunity to smallpox. In 1798, the first smallpox **vaccine** was developed. Since then, a plethora of vaccines have been developed.

In 1928, Alexander Fleming had discovered the first antibiotic, penicillin, but it took over a decade before penicillin was introduced as a treatment for bacterial infections. This was possible through the work of Florey and Chain who managed to efficiently purify the antibiotic and scale-up production. The introduction of penicillin marked the beginning of the so-called “golden era” of antibiotics.

2. Learning Outcomes

a. Describe the specific (adaptive) and non-specific (innate) immune systems including active and passive, natural and acquired immunity.

e. Discuss how vaccination can control disease (e.g. in the eradication of small pox), limited to vaccination stimulates immunity without causing the disease and vaccination of a high enough proportion of the population can break the disease transmission cycle.

f. Discuss the benefits and risks of vaccination.

g. Explain how viruses, including influenza and HIV, cause diseases in humans through the disruption of host tissue and functions (e.g. HIV and T helper cells, influenza and epithelial cells of the respiratory tract).

h. Explain the mode of transmission and infection of bacterial pathogens, using *Mycobacterium tuberculosis* as an example.

i. Describe the modes of action of antibiotics, including penicillin, on bacteria.

3. References

Judith Owen, Jenni Punt, Sharon Stranford. Kuby Immunology, 7th Edition.

Murphy KM, P Travers, M Walport (Eds.) Janeway’s Immunobiology. 8th Edition.

Pommerville, Jeffrey C. Alcamo’s Fundamentals of Microbiology: Body Systems.

Contents

4. Vaccination

Notes to self

The knowledge of immunological memory has been used in medical practice to improve human health and survival through the practice of vaccination.

The type of immunity described so far occurs during the course of an infection, when an immune response to antigens activates lymphocytes and **antibodies** are **produced by plasma cells of the infected individual**. This is known as **natural active immunity**.

Natural immunity is immunity gained by being infected by pathogen (active) or by receiving antibodies from the mother across the placenta or in breast milk (passive).

Differences between active immunity and passive immunity:

	Active Immunity	**Passive Immunity**
Antibody production	Antibodies are **produced by the individual's own immune system** in response to antigens introduced naturally or artificially.	Antibodies are **transferred to the recipient** without the participation of the recipient's immune system. Examples: (1) Fetus receives IgG antibodies from mother through transfer across the placenta. (2) Newborn receives antibodies such as IgA from mother through the breast milk.
Duration of immunity	Immunity conferred is **long-lasting** since **memory cells** are formed in the individual after primary immune response to antigen.	Immunity conferred is **short-lived** since there is **no formation of memory cells**.

Notes to self

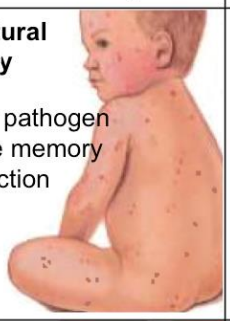
	Active immunity	Passive immunity
Natural immunity	**Active, natural immunity** infected by pathogen to stimulate memory cells production 	**Passive, natural immunity** • fetus receives antibodies through placenta • newborn receives antibodies from breast milk 
Artificial immunity	**Active, artificial immunity** receive vaccination to stimulate memory cells production 	**Passive, artificial immunity** receive anti-serum with antibodies from another host 

Fig. 1:. Overview of the 4 different types of immunity and their corresponding examples.

Vaccination is the intentional **administration of a harmless form of a pathogen** in order to **induce a specific adaptive immune response** that protects the individual against later exposure to the pathogen due to production of memory cells. The individual **should not develop disease symptoms**.

Vaccination is an **artificial active immunity** since the immune response is activated artificially by introducing antigens into the body to initiate primary immune response.

Vaccination uses the property of **immunological memory** to provide long-lasting protection against infectious diseases.

When exposed to the actual pathogen, the memory cells trigger a secondary immune response that is faster and stronger.

Notes to self

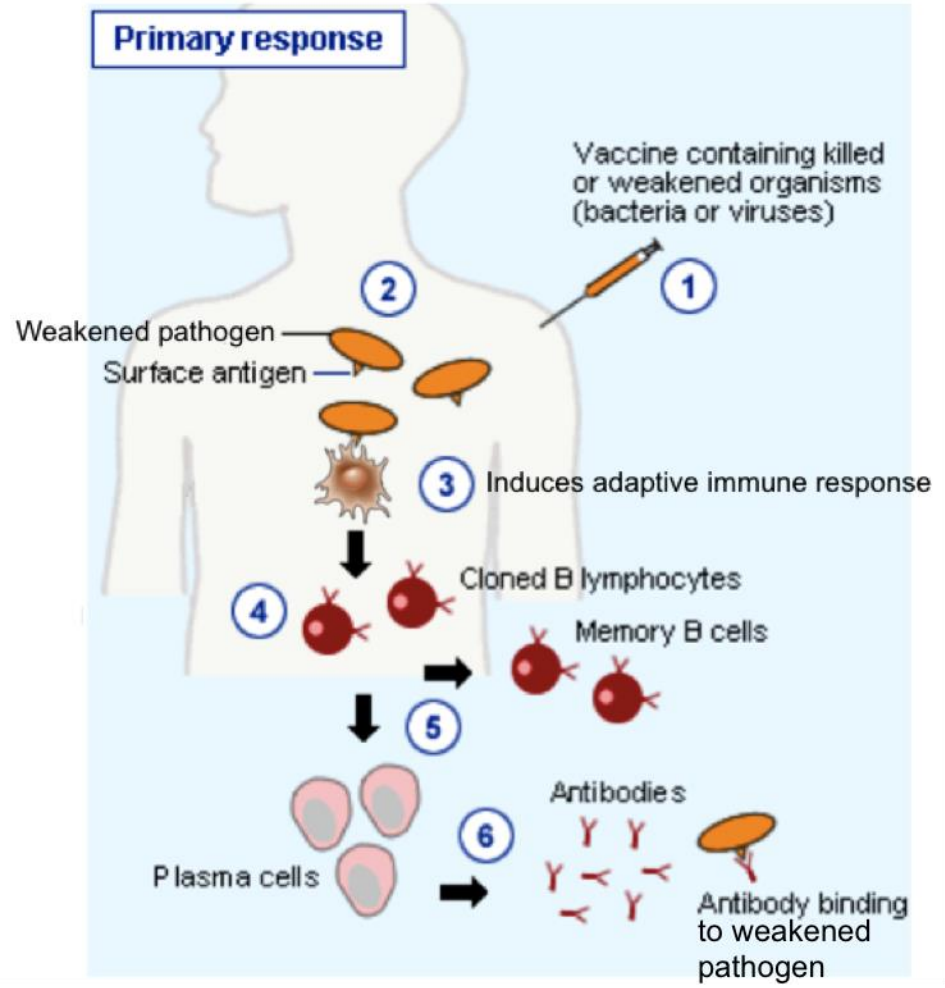


Fig. 2: Events that happen after vaccination.

The events that happen after vaccination (Fig. 2):

(1) A person is vaccinated against a specific disease. The vaccine contains either the attenuated or weakened form of pathogen or the killed pathogen.
(2) The modified pathogen is no longer able to cause disease, but it can still retain immunogenic effect (i.e. ability to elicit immune response) because a characteristic surface antigen of the pathogen is still retained and can be recognised by antigen presenting cells.
(3) Adaptive immune response occurs. Naïve B and T lymphocytes are activated to become effector lymphocytes.
(4) The B lymphocyte with the specific BCR that is complementary in shape to the antigen undergoes clonal expansion by dividing repeatedly by mitosis to form genetically identical B lymphocytes which become either
(5) memory cells or
(6) antibody-secreting plasma cells.

Vaccination can prevent disease by generating a rapid and more intense immune response. (Fig. 3)

Notes to self

(1) If the previously vaccinated individual is exposed to the virulent pathogen,
(2) memory cells will quickly recognize the surface antigen of the pathogen.
(3) Memory cells rapidly undergo clonal expansion and
(4) they develop into antibody secreting plasma cells.
(5) These plasma cells produce a large number of antibodies which are able to bind and inactivate the virulent pathogen to prevent them from infecting the healthy host cells in the individual.

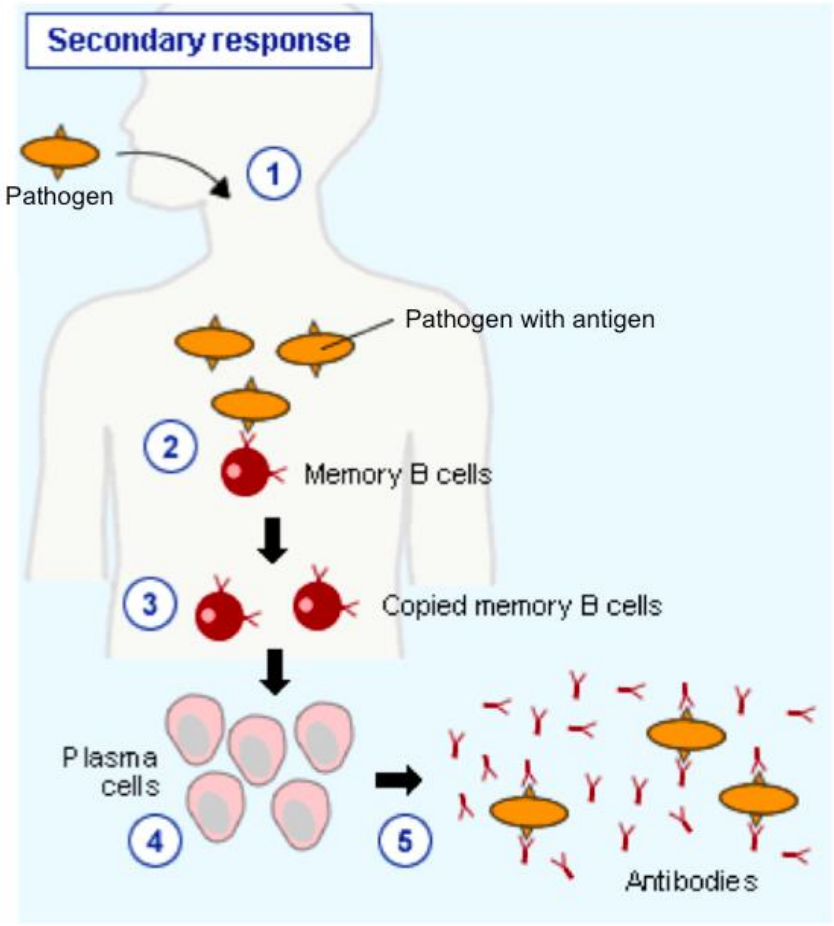


Fig. 3: Vaccination can prevent disease by generating a rapid and more intense immune response.

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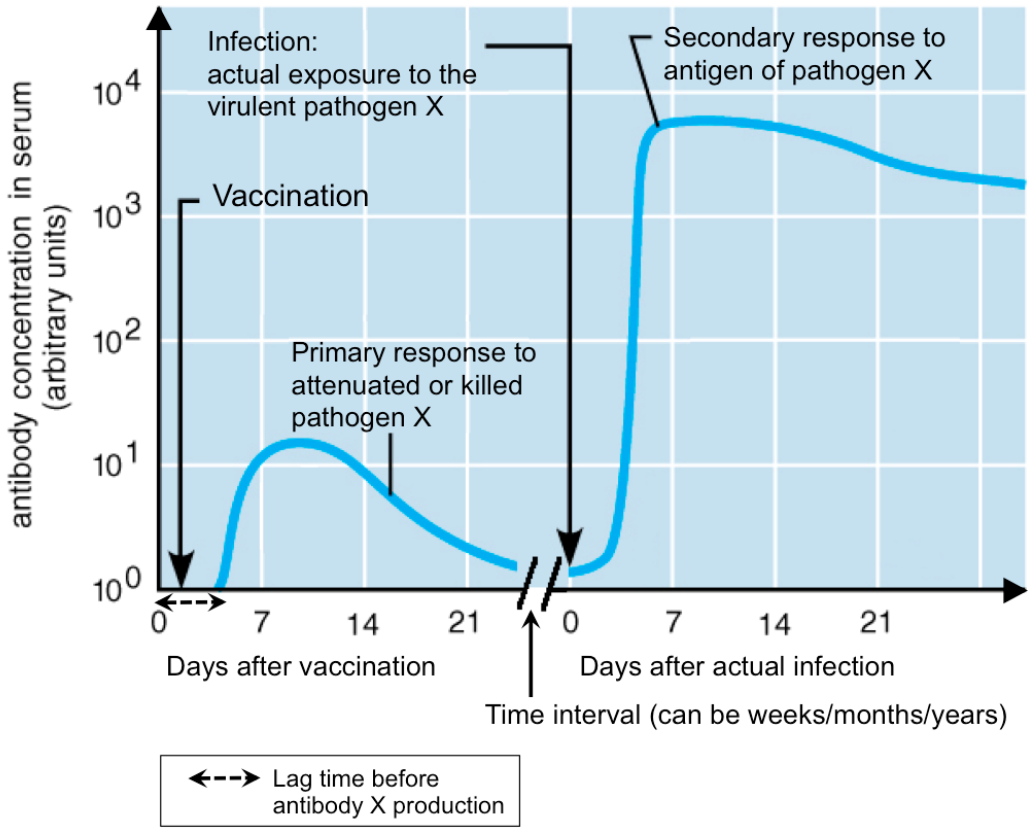

Fig. 4: Concentration of serum antibody following vaccination (primary response) and infection by virulent pathogen (secondary exposure). (Modified: Human Anatomy and Physiology, Elaine Marieb)

Types of Vaccine

A vaccine is a preparation of immunogenic material used to induce immunity against pathogenic organisms. Three types of vaccines:

1. Live, attenuated vaccine
2. Inactivated vaccine
3. Toxoid vaccine

Live, attenuated vaccine

Attenuation refers to weakening of the pathogenic bacteria or virus by making it less virulent.

Advantages:

- A live, attenuated vaccine is the closest thing to a natural infection, thus it will elicit **strong immune response with just a low dosage** and confer **longer-term protection**.

Disadvantages:

- Possibility of their **reversion to the virulent form** by mutation and cause disease.
- **Need to be refrigerated** to stay potent.

Notes to self

Inactivated vaccine

Inactivation involves killing the pathogenic bacteria or virus with chemicals, heat or radiation.

Advantages:

- **Will not revert to the virulent form** and cause disease.
- Usually **do not require refrigeration**, and it can be easily stored and transported in a freeze-dried form to make inactivated vaccine accessible to people in developing country.

Disadvantages:

- Elicit **weaker immune response**, thus may need several doses (i.e. booster shots) to maintain protection.

Toxoid vaccine

Toxoid is a **chemically modified toxin** from pathogenic bacteria such that it is no longer toxic but is still to elicit immune response.

It is used when bacterial toxin is the main cause of illness. Vaccination with toxoid induces anti-toxoid antibodies which can bind the bacterial toxins and neutralise the toxins.

Advantage:

- Does not contain any pathogens thus there is no risk of reverting to virulent form.

Disadvantage:

- Limited use when bacterial toxin is the main cause of illness but not all pathogenic bacteria produce toxins.

Benefits & Risks of Vaccination

Benefits

Some diseases may be completely eradicated by vaccination. e.g. Smallpox has been eradicated by vaccination, reducing human suffering and future costs of treatment.

Vaccines protect individuals against diseases:

- Deaths due to diseases can be prevented.
- Long-term disabilities such as infertility, deafness and blindness due to the diseases can be prevented.

If there are sufficient individuals in a population who are immune to an infectious agent, transmission of the disease is prevented due to the low chance of a susceptible individual contacting an infected individual. This is known as **herd immunity**. If the pathogen is highly infectious i.e. one infected individual can rapidly infect several non-immune individuals, a large proportion of the population needs to be immune to maintain herd immunity.

Risks

Notes to self

However, the immunity developed after vaccination may not be as effective as natural immunity due to a real infection.

There are a variety of concerns regarding the use of vaccines:

- While live, attenuated vaccines are more effective than inactivated vaccines, they pose the risk of reversion to virulence to cause disease.

- Some vaccines may cause side effects that cause a different illness than the disease the vaccines are designed to prevent. e.g. possible autism from certain vaccines.

  Most side effects from vaccination are mild, such as soreness, swelling, or redness at the injection site. Some vaccines are associated with fever, rash, and achiness. Serious side effects are rare, but may include seizure, encephalitis, life-threatening allergic reaction or even death.

5. Pathogens & Diseases

Pathogens are microorganisms **invading the body to cause diseases**. The 5 classes of pathogens are viruses, bacteria, fungi, protozoa and worms.

Only relatively few, and not all, bacteria or fungi are parasitic and pathogenic. All viruses are obligate parasites, and they can be pathogenic.

Pathogens of all classes must have specialised mechanisms for crossing cellular and biochemical barriers of their host organisms and for evading destruction by the host innate and adaptive immune responses. Pathogens can then replicate using host resources.

A **disease** is an abnormal condition of an organism or impairment to the normal functioning of an organism as a result of the presence of pathogens in the organism.

Many of the symptoms and signs that are associated with infectious diseases are direct manifestations of the host's immune responses in action. e.g. Swelling and redness at the site of infection and the production of pus (mainly dead white blood cells) are hallmarks of bacterial infection. These signs are direct result of cells of the immune system attempting to destroy the invading bacteria.

Examples of pathogen and the disease the pathogen causes:

Name of pathogen	Type of pathogen	Name of disease
influenza virus	virus	influenza
human immunodeficiency virus (HIV)	virus	acquired immunodeficiency syndrome (AIDS)
dengue fever virus	virus	dengue fever
Mycobacterium tuberculosis	bacterium	tuberculosis
Plasmodium falciparium	protozoan	malaria

Some viruses escape elimination by the immune response by establishing **latent** (hidden) **infection**. These viruses remain in the host even after disease symptoms disappear and are generally undetectable during the latent periods. The disease may be periodically reactivated by various stimuli.

Notes to self

a. Pathogenicity of Influenza Virus

Target cells:

- epithelial cells of the respiratory tract
- Virus binds to the sialic acid receptor found on the epithelial cell membrane.

Mode of transmission:

- droplets of moisture from lungs of infected persons or from infected bird droppings

The disease:

- Once the virus settles on the mucous membrane lining the nose, pharynx, trachea and bronchi, the neuraminidase enzyme on the surface of the viruses helps them to penetrate the mucoproteins in the mucus layer. The mucoproteins are glycoproteins in nature.
- The haemagglutinin, a glycoprotein on the viral envelope, then helps the virus bind to specific receptors on cell membrane of the epithelial cell lining the respiratory tract. Eventually, the virus penetrates into these host cells. Once inside, the virus replicates within them.
- The incubation period is around 24 to 48 hours, after which the infected epithelial cells are destroyed. These lead to inflammation and the build-up of dead epithelial cells in the airways, causing the symptoms of influenza like running nose and scratchy throat to appear.
- Weakening of the epithelial layer caused by viral replication can make the respiratory passage more susceptible to secondary bacterial infections leading to diseases like pneumonia which can be fatal.

Treatment:

- There is no treatment for most people who develop influenza. However, bed rest, and perhaps the administration of aspirin or paracetamol to alleviate headaches and fever; is the only helpful means towards recovery.
- Antibiotics are administered to prevent secondary bacterial infection like pneumonia.
- Vaccinations against influenza are also sometimes administered. The influenza vaccine contains purified and inactivated material from the three common influenza viral strains.
- Antiviral drugs such as oseltamivir (trade name Tamiflu) and zanamivir (trade name Relenza) are neuraminidase inhibitors that are designed to halt the spread of the virus in the body. However, these drugs are more suited towards the Influenza A and Influenza B strains. The antiviral drugs amantadine and rimantadine are designed to block a viral ion channel (M2 protein) and prevent the virus from infecting cells. These drugs are effective against Influenza A but not against Influenza B.

b. Pathogenicity of HIV

Notes to self

Target cells:

- T helper cells and macrophages of the immune system
- Virus binds to the CD4 receptor found on the host cell membrane.

Mode of transmission:

- Transmitted primarily through unprotected sexual contact or by exposure to infected blood and blood products.
- It can also be transmitted from mother to child either via the placenta, during childbirth or breastfeeding.

The disease:

- Once the virus enters the blood stream, its primary targets are macrophages (a large phagocytic cell) and T helper cells (a type of lymphocyte responsible for the coordination of an immune response to infection).
- The HIV virus has a very strong affinity for and binds to CD4, a surface protein of T helper cells. As the HIV infects more and more T helper cells, levels of T helper cells lower as the infected cells are destroyed.
- The macrophages may survive HIV infection (because they are not lysed by the virus) and may thus act as reservoirs.
- HIV may be passed from cell to cell in an infected individual, or it may be transmitted via body fluids to another person, while still remaining undetected.
- The virus mutates at a very high rate during replication resulting in altered proteins on the surface of the virus. In this way, the virus prevents recognition and elimination by the immune system allowing it to evolve rapidly within the body.
- The increasing loss of T helper cells leads to impaired immune responses in the affected individual who then becomes increasingly susceptible to opportunistic diseases. 'Full-blown AIDS' occurs when the infections become unmanageable. The immuno-suppression becomes worse, usually with fatal results. Death usually results from secondary infections.

Treatment:

- Due to the high rates of virus productions and mutation rate of the virus, treatment of HIV infection generally includes administration of 3 agents in combination. Sustained treatment results in suppression of viral replication, dramatically increasing life expectancy of HIV-infected individuals.
- Currently, there are 24 approved retroviral drugs which can be used to treat HIV infection. They include reverse transcriptase inhibitors, protease inhibitors, integrase inhibitors and entry inhibitors. The first three enzyme inhibitors work by inhibiting their respective enzymes. Entry inhibitors work by blocking interaction between the HIV envelope and CD4 or the co-receptor, or by preventing fusion of the viral and host cell membranes, thus blocking entry of HIV into the cells.

c. Pathogenicity of *Mycobacterium tuberculosis*

Notes to self

Mycobacterium tuberculosis (*M. tuberculosis*) which is an obligate aerobe. Obligate aerobe is a microorganism that requires oxygen for survival and growth. Without oxygen, aerobic respiration cannot occur and obligate aerobe cannot survive.

Target organ:

- Primary infection is usually in the **lungs** to cause pulmonary tuberculosis (TB).
- Other parts of the body e.g. kidneys, spine can also be infected to cause extrapulmonary tuberculosis (TB).

Mode of transmission:

- The bacteria are transmitted from person to person in **fine, aerosol droplets** when an infected person with the active TB disease sneezes or coughs and an uninfected person inhales the droplets.
- Thus, tuberculosis is an **airborne disease**.
- TB spreads rapidly among people living in overcrowded conditions.

The disease:

- The minimum infectious dose for lung infection is around 10 bacterial cells.
- Once inside the lungs, **alveolar macrophages phagocytose the bacteria** and form phagosomes containing *M. tuberculosis*. (Fig. 5)
- Inside the phagosome, *M. tuberculosis* **inhibits the fusion of the phagosome with lysosomes**. No phagolysosome is formed and no lysosomal enzymes are available to kill the bacteria.
- *M. tuberculosis* **survives** and continue to **multiply** inside the macrophages. (Fig. 6)
- A **tubercle**, a tight ball-like formation by clustering cells such as macrophages, is formed. (Fig. 7) At the center of the tubercle, cell death by **necrosis** occurs. Cell death by necrosis, unlike apoptosis, involves the rupturing of cell membrane and releasing of cell content to the surrounding.
- The disease may be arrested at this stage and remain latent for several years. People with this latent infection do not spread the disease to others.
- Some people become infected and develop TB after a few weeks. The tubercle cavity enlarges to form an air-filled cavity for the aerobic *M. tuberculosis* to multiply outside the macrophages.
- The **tubercle ruptures**, allowing *M. tuberculosis* to **spill into a bronchiole** and spread throughout the lungs. This results in development of a productive cough that facilitates aerosol spread of infectious bacteria. (Fig. 8)
- The lungs will be progressively destroyed by the formation of cavities due to the rupture of tubercles.
- TB is often the first opportunistic infection to strike HIV-positive people. HIV infection may reactivate dormant infections of *M. tuberculosis* which may have been present in the person from childhood. If the HIV-positive person is previously uninfected by *M. tuberculosis*, he/she will be more susceptible to infection by the bacteria.

Notes to self

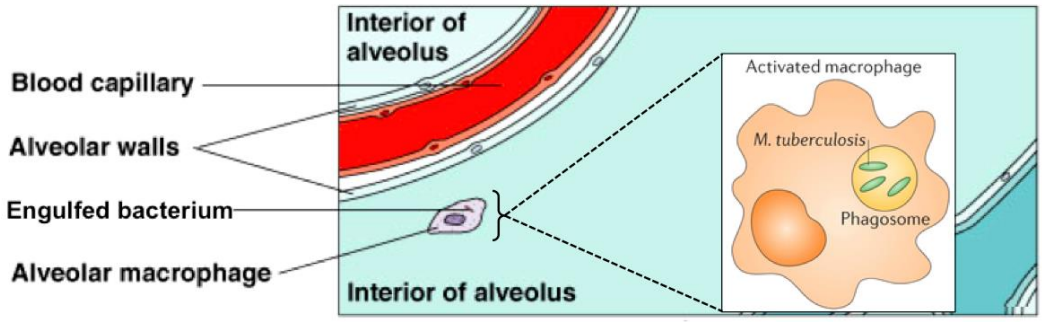

Fig. 5: Alveoli of the lung contain macrophages that phagocytose *M. tuberculosis* that reach the alveoli. (Modified: Microbiology An Introduction 8th Edition)

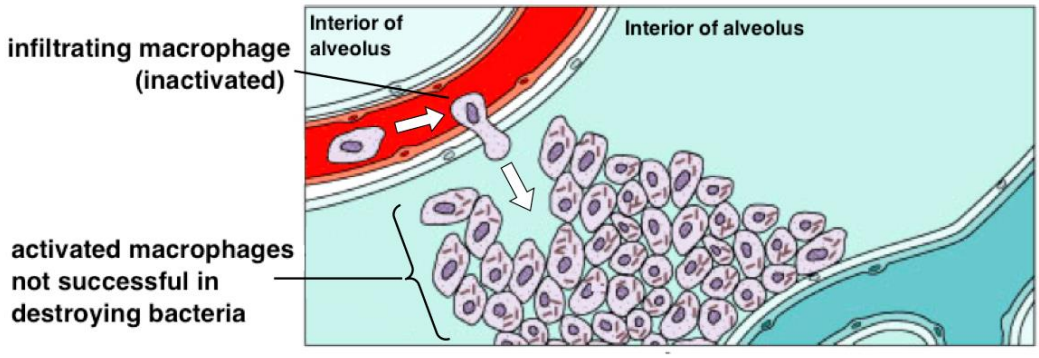

Fig 6: *M. tuberculosis* can survive and multiply in macrophages and more macrophages are brought to the infected alveolus.

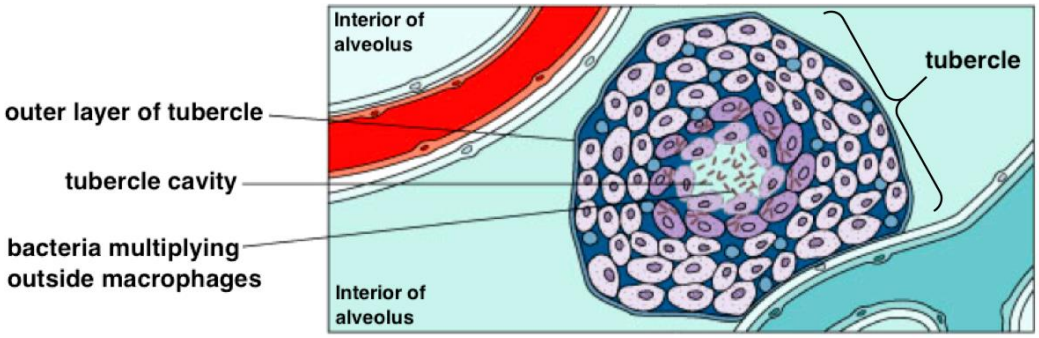

Fig. 7: At the center of tubercle macrophages die, releasing *M. tuberculosis* into the cavity.

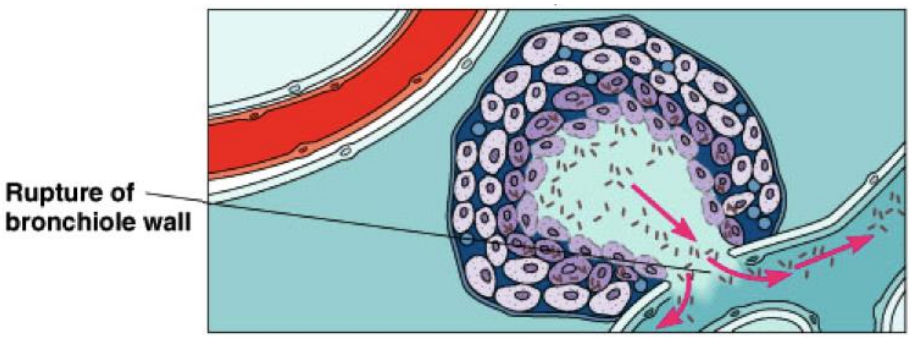

Fig. 8: Tubercle ruptures, releasing *M. tuberculosis* into the bronchiole and thus disseminating throughout the lungs and into the circulatory and lymphatic systems.

Treatment of TB:

Notes to self

About 6 months of daily treatment with a **combination of at least two antibiotics**. If the patient stops taking the prescribed antibiotics before the complete course, or if a dose is skipped, the TB infection may become resistant to the antibiotics. This is potentially serious as it can be difficult to treat and will require a longer course of treatment.

A combination of antibiotics is used during treatment to minimise the risk of developing resistance to the antibiotics, and also to achieve an additive effect against the bacteria.

Mechanism of action of antibiotics used for TB treatment:

- Inhibits synthesis of mycobacterial cell wall e.g. antibiotic isoniazid.
- Inhibits RNA synthesis during transcription e.g. antibiotic rifampin interferes with prokaryotic RNA polymerase.

Prevention of TB:

A vaccine against TB, Bacillus Calmette-Guérin (BCG) vaccine, is prepared from a strain of attenuated live *Mycobacterium bovis*, a bovine tuberculosis bacillus. It is often used to prevent the spread of TB among children.

6. Mode of Action of Antibiotics

Antibiotics are **natural substances obtained from microorganisms** such as certain fungi and bacteria. They **inhibit the growth** of bacteria or **kill** the bacteria by disrupting the metabolism of the prokaryotic cells.

A **bacteriostatic** antibiotic is one that is able to inhibit the growth or cell division of bacteria.

A **bactericidal** antibiotic is one that is able to kill bacteria when the bacteria are in the process of undergoing cell division by binary fission.

Question:
Why antibiotics can inhibit or kill bacterial cells but have little or no effect on human cells?

Answer: An effective antibiotic specifically disrupts the metabolism of the disease-causing bacteria but not the metabolism of mammalian host tissues because prokaryotic and eukaryotic cells use different metabolic machineries e.g. different ribosomal subunits.

Question: Why antibiotics cannot be used to treat viral diseases?

Answer: Antibiotics do not affect viruses as a virus lacks cell structure and has no metabolism of its own to be disrupted by antibiotics. Viruses use metabolic machineries of host cells that are not affected by antibiotics and can continue their viral reproductive cycles.

Three major prokaryotic metabolic pathways that antibiotics usually disrupt:

Notes to self

1. Cell wall synthesis
2. Protein synthesis (translation)
3. Nucleic acid synthesis

Antibiotics that **disrupt cell wall synthesis**

The best target for antibiotics is the bacterial cell wall because human cells do not have cell wall.

Most bacterial cell walls contain **peptidoglycan**. Peptidoglycan, a structure unique to bacterial cells, is essential for maintaining the structural integrity of the bacterial cells. Thus, the bacterial cell wall has a high internal osmotic pressure, and the rigid cell wall maintains the shape of the bacterium.

Penicillin (Fig. 9) is an example of an antibiotic that inhibits bacterial cell wall synthesis i.e. disrupts peptidoglycan synthesis. What is the action of penicillin on bacteria? (Fig. 10)

- Penicillin acts as a competitive inhibitor and binds to the active site of transpeptidase.
- As a result, there is inhibition to the formation of cross-links between adjacent chains.
- Bacterial cell wall becomes weakened when bacterial cells carry out division.
- When bacteria take in water by osmosis, the increased turgor pressure against the weakened cell wall causes the bacteria to swell and lyse.
- Penicillin is only effective when bacterium is growing or making new cell wall.

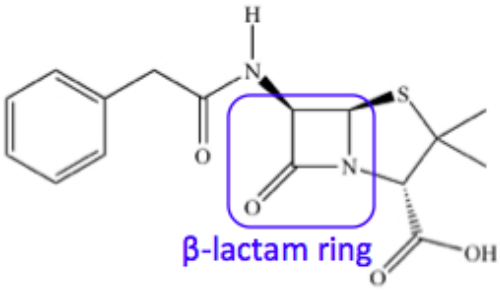

Fig. 9: Structure of penicillin, a β-lactam antibiotics. Members of β-lactam antibiotics are characterised by their four-membered, nitrogen containing β-lactam ring structure.

Notes to self

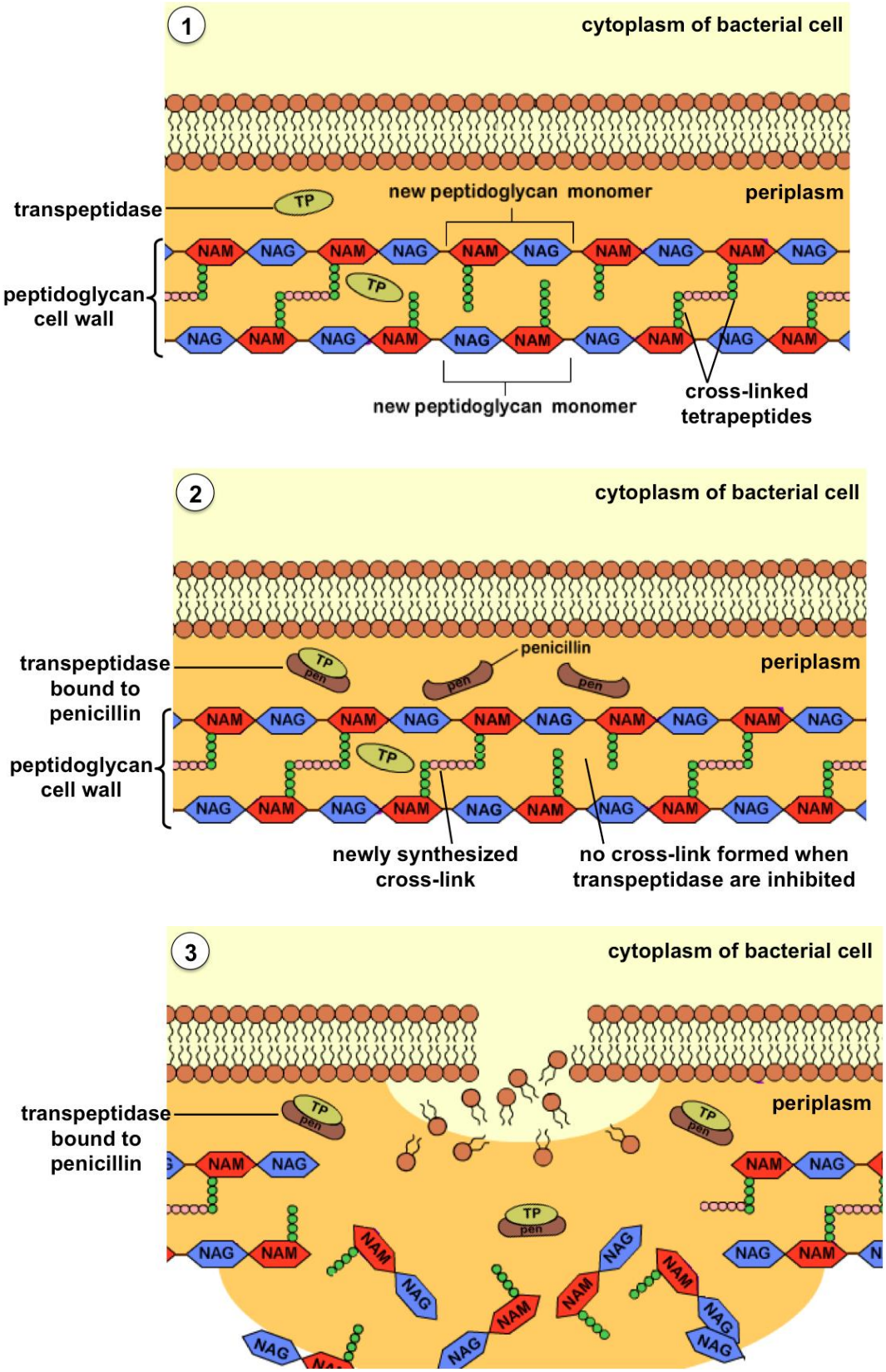

Fig. 10: Bactericidal effect of penicillin. (1) As new monomers are linked to existing rows of peptidoglycan during bacterial cell wall synthesis, transpeptidase enzymes catalyse the formation of cross-links between peptides that are attached to N-acetylmuramic acid (NAM) residues of adjacent chain. These cross-links gives peptidoglycan its high tensile strength. (2) Penicillin binds to transpeptidase enzymes to inhibit the formation of cross-links between adjacent chains, weakening bacterial cell wall of dividing bacterial cells. (3) Osmotic lysis happens to dividing bacterial cells since their cell wall synthesis is inhibited.

Antibiotics that **disrupt protein synthesis**

Notes to self

- Ribosomes of prokaryotes are 70S while ribosomes of eukaryotes are 80S.
- Streptomycin is an example of an antibiotic that inhibits protein synthesis by **binding to the small subunit** (30S) of the bacterial ribosome such that the **initiator tRNA cannot bind to the small subunit**.
- Tetracycline is another example of an antibiotic that inhibits protein synthesis by **blocking aminoacyl-tRNA from attaching to the A site** of bacterial ribosome.

Antibiotics that **disrupt nucleic acid synthesis**

- Rifampin inhibits RNA synthesis by binding to bacterial RNA polymerase thus **preventing transcription**.