

IMMUNOLOGY

- Study of how an organism defends itself against infection by microorganism.
- Upon invasion of the body by microorganisms (bacteria), white blood cells engulf such bacterial cells and digest it in an immune response process called phagocytosis.
- *Phagocytosis* is the ability of a cell to engulf and digest solid materials by the use of “*pseudopods*” or “false feet.”
- Phagocytosis was discovered in 1880 by Russian zoologist Elie Metchnikoff, who was one of the first scientists to study immunology.
- Metchnikoff studied the body’s defense against disease-causing agents and invading microorganisms. He discovered that leukocytes (White blood cells) defended the body by engulfing and eating the invading microorganism

DRUGS:

- Antibiotics are drugs that contain one or more antimicrobial agents that combat bacteria.
- An antimicrobial agent is a substance that specifically inhibits and destroys the attacking microorganism.
- One of the most commonly used antimicrobial agents is penicillin.
- *Penicillin* is made from *Penicillium*, which is a mold that secretes materials that interfere with the synthesis of the cell walls of bacteria causing “lysis” or destruction of the cell wall, and kills the invading microorganism.

HISTORY OF MICROBIOLOGY

The microscope

i. Zacharias Janssen

- In 1590, Zacharias Janssen developed the first compound microscope in Middleburg, Holland (1590).
- Janssen’s microscope consisted of three tubes. One tube served as the outer casing and contained the other two tubes. At either ends of the inner tubes were lenses used for magnification.
- Janssen’s design enabled scientists to enlarge the image of a specimen three and nine times the specimen’s actual size.

ii. Robert Hooke

- Robert Hooke, an English scientist, popularized the use of the compound microscope in 1665 when he placed lenses over slices of cork and viewed little boxes that he called cells.

- It was his discovery that led to the development of cell theory in the nineteenth century by Mathias Schleiden, Theoder Schwann, and Rudolf Virchow.
- Cell theory states that all living things are composed of cells.

iii. Antoni van Leeuwenhoek

- Hooke's experiments with a crude microscope inspired Antoni van Leenwenhoek to further explore the micro world.
- Van Leeuwenhock, an amateur lens grinder, improved Hooke's microscope by grinding lenses to achieve magnification.
- His microscope required one lens. With his improvement, van Leeuwenhock became the first person to view living microorganisms, which he called Animalcules.
- This discovery took place during the 1600s, when scientists believed that organisms generated spontaneously and did not come from other organisms. This sounds preposterous today; however, back then scientists were just leaning that a cell was the basic component of an organism.

Origin of Organisms

i. Francesco Redi

- In 1668, Italian physician Francesco Redi developed an experiment that demonstrated that an organisms did not spontaneously appear.
- He filled jars with rotting meat. Some jars he sealed and other he left opened. Those that were open eventually contained maggots, which is the larval stage of the fly. The other jars did not contain maggots because flies could not enter the jar to lay eggs on the rotting meat.
- His critics stated that air was the ingredient required for spontaneous generation of an organism.
- Air was absent from the sealed jar and therefore no spontaneous generation could occur, they said Redi repeated the experiment except this time he placed a screen over the opened jars.
- This prevented flies from entering the jar. There weren't any maggots on the rotting meat.
- Until that time scientists did not have a clue about how to fight disease. However, Redi's discovery gave scientists an idea.
- They used Redi's findings to conclude that killing the microorganisms that caused a disease could prevent the disease from occurring.
- A new microorganism could only be generated by another microorganism when it underwent a reproductive process. Kill that microorganism and you will not have new microorganisms, the theory went – you could stop the spread of the disease. Scientists called this the Theory of Biogenesis.
- The Theory of Biogenesis states that a living cell is generated from another living cell.

ii. Louis Pasteur

- Theory of Biogenesis disproved spontaneous generation
- Spontaneous generation was hotly debated among the scientific community
- In 1861 Louis Pasteur, a French scientist, resolved the issue once and for all.
- Pasteur showed that microorganisms were in the air. He proved that sterilized medical instruments became contaminated once they were exposed to the air.
- Pasteur came to this conclusion by boiling beef broth in several short-necked flasks. Some flasks were left open to cool. Other flasks were sealed after boiling.
- The opened flasks became contaminated with microorganisms while no microorganisms appeared in the closed flasks. Pasteur concluded that airborne microorganisms had contaminated the opened flasks.
- In a follow-up experiment, Pasteur placed beef broth in an open long-necked flask. The neck was bent into an S-shape. Again he boiled the beef broth and let it cool. The S-shaped neck trapped the airborne microorganisms.
- The beef broth remained uncontaminated even after months of being exposed to the air. The very same flask containing the original beef broth exists today in Pasteur Institute in Paris and still shows no sign of contamination. Pasteur's experiments validated that microorganisms are not spontaneously generated.
- Based on Pasteur's findings, Pasteurization, one of the best-known sterilization techniques, was developed and named after Pasteur.
- Pasteurization kills harmful microorganisms in milk, alcoholic beverages, and other foods and drinks by heating it enough to kill most bacteria that cause spoilage.

iii. **John Tyndall and Ferdinand Cohn**

- John Tyndall and Ferdinand Cohn made one of the most important discoveries in sterilization the late 1800s.
- They discovered that some microorganisms are resistant to certain sterilization techniques.
- Until their discovery, scientists believed no microorganisms could survive boiling water, which was a widely accepted method of sterilization. This notion was wrong.
- Some thermophiles (type of bacteria) resisted heat and could survive a bath in boiling water. This means that there was not one magic bullet that killed all harmful microorganisms.

Germ theory

i. **Rober Koch**

- Koch made some observations on the disease caused *Bacillus anthracis* called anthrax.
- Based on his findings, Koch developed the Germ Theory.

- The Germ Theory states that a disease-causing microorganism should be present in animals infected by the disease and not in healthy animals. The microorganisms can be cultivated away from the animal and used to inoculate a healthy animal. The healthy animal should then come down with the disease. Samples of a microorganism taken from several infected animals are the same as the original microorganism from the first infected animals.
- Four steps used by Koch to study microorganisms are referred to as Koch's Postulates.
- Koch's Postulates state:
 1. The microorganism must be present in the diseased animals and not present in the healthy animal.
 2. Cultivate the microorganism away from the animal in a pure culture.
 3. Symptoms of the disease should appear in the healthy animal after the healthy animal is inoculated with the culture of the microorganisms.
 4. Isolate the microorganism from the newly infected animal and culture it in the laboratory. The new culture should be the same as the microorganism that was cultivated from the original diseased animal.
- Koch's work with anthrax also led to the development of technique for (culture) growing microorganisms.
- He eventually used a gelatin surface (solid phase) to cultivate microorganisms.
- Gelatin inhibited the movement of microorganisms. As they reproduced, they remained together, forming a colony (a mound of cells) that made them visible without a microscope.
- Gelatin was replaced with agar that is derived from seaweed and still used today.
- Richard Petri improved on Koch's cultivating technique by placing the agar in a specially designed disk that was later called the Petri dish which is still used today.

Vaccination

- *Variola* virus was once the most feared villain in the late 1700s.
- The variola virus causes smallpox.
- Cows were also susceptible to a variation of variola called cowpox.
- Milkmaids who tended to infected cows contracted cowpox and exhibited immunity to the smallpox virus.

i. Edward Jenner

- Edward Jenner, an English physician, discovered something very interesting about both smallpox and cowpox in 1796.
- Those who survived smallpox never contracted smallpox again, even when they were later exposed to someone who was infected with smallpox.
- Milkmaids who contracted cowpox never caught smallpox even though they were exposed to smallpox.

- Jenner took scrapings from a cowpox blister found on a milkmaid and, using a needle scratched the scarring into the arm of James Phipps, an 8-year-old.
- Phipps became slightly ill when the scratch turned bumpy. Phipps recovered and was then exposed to smallpox. He did not contract smallpox because his immune system developed antibodies that could fight off variola.
- Jenner's experiment led to the discovery of vaccination
- Vaccination which is an extension of the Latin word vacca (cow). The person who received the vaccination became immune to the disease-causing microorganism.

ii. Elie Metchnikoff

- Elie Metchnikoff, a nineteenth-century Russian zoologist, was fascinated by Jenner's work with vaccinations.
- Metchnikoff wanted to learn how our bodies react to vaccination by exploring our body's immune system.
- He discovered that white blood cells (leukocytes) engulf and digest microorganisms that invade the body. He called these cells phagocytes, which means "cell eating".
- Metchnikoff was one of the first scientists to study the new area of biology called immunology, the study of the immune system.

Killing the Microorganisms.

Ignaz Semmelweis

- During the late 1800s Hungarian physician Ignaz Semmelweis observed a dramatic decline in childbirth fever when physicians used antiseptic techniques when delivering babies.
- Infections become preventable through the use of antiseptic techniques.

ii. Joseph Lister

- Joseph Lister, an English surgeon, developed one of the most notable antiseptic techniques.
- During surgery he sprayed carbolic acid over the patient and then bandaged the patient's wound with carbolic acid-soaked bandages.
- Infection following surgery dramatically dropped when compared with surgery performed without spraying carbolic acid.
- Carbolic acid, also known as phenol was one of the first surgical antiseptic.

iii. Paul Ehrlich

- Antiseptics prevented microorganisms from infecting a person, but scientists still needed a way to kill microorganisms after they infected the body.
- At the turn of the nineteenth century, Paul Ehrlich, a German chemist, discovered the magic bullet.

- Ehrlich blended chemical elements into a concoction that, when inserted into an infected area, killed microorganisms without affecting the patient.
- Today we call Ehrlich's concoction a drug. Ehrlich's innovation has led to chemotherapy using drugs that are produced by chemical synthesis.

iv. Alexander Fleming

- Following Ehrlich's findings, scientists attempted to find drugs that could make infected patients well again.
- One of the most striking breakthroughs came in 1929 when Alexander Fleming discovered *Penicillium notatum*, the organism that synthesizes penicillin.
- *Penicillium notatum* is a fungus that kills the *Staphylococcus aureus* microorganism and similar microorganisms.
- Fleming grew cultures of *Staphylococcus aureus*, a bacterium, in the laboratory. He was also conducting experiments with *Penicillium notatum*, a mold. By accident the *Staphylococcus aureus* was contaminated with the *Penicillium notatum*, causing the *Staphylococcus* to stop reproducing and die.
- *Penicillium notatum* became one of the first antibiotics.
- An antibiotic is a substance that kills bacteria.

PREPARING SPECIMEN FOR OBSERVATION UNDER A LIGHT COMPOUND MICROSCOPE

- Two ways to prepare a specimen for observation under a light compound microscope;
- *Smear* and *wet mount*.

Smear

- A process where a specimen is spread on a slide. A smear is prepared using the heat fixation method.
1. Use a clean glass slide.
 2. Take a smear of the culture. (The microorganisms are spread over the glass slide).
 3. Place the live microorganism on the glass slide by smearing it onto the glass.
 4. The slide is air dried then passed over a Bunsen burner about three times.
 5. The heat causes the microorganisms to adhere to the glass slide. This is known as fixing the microorganisms to the glass slide.
 6. Stain the microorganisms with an appropriate stain.

Wet Mount

A wet mount is a preparation process where a live specimen in culture fluid is placed on a concave glass slide or a plain glass slide. The concave portion of the glass tube forms a cup-like shape that is filled with a thick, syrupy substance, such as carboxymethyl cellulose. The microorganism is free to move about within the fluid, although the viscosity of the substance

slows its movement. This makes it easier for you to observe the microorganism. The specimen and the substance are protected from spillage and outside contaminants by a glass cover that is placed over the concave portion of the slide.