Al-Mustaqbal University College
Dept. Medical Lab. Techniques
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By Prof. Dr. Habeeb S. Naher
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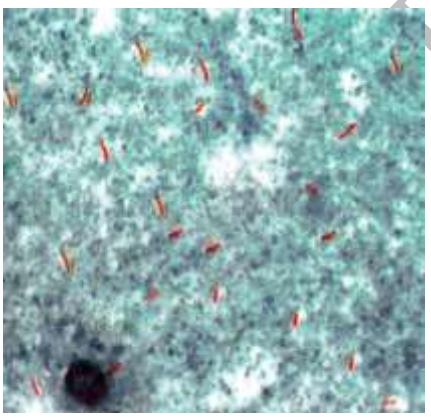
Lecture-11: Genus Mycobacterium, (*Mycobacterium tuberculosis*)
Tuberculosis disease (TB disease)

Cell **morphology**: *M. tuberculosis* is; **1- a slender**, **straight or slightly curved rod with rounded ends**, **in pairs** or as **small bundles**. **2-** They are **non-motile, non-sporing, non-capsulated and acid-fast. 3- TB bacilli are gram positive, but difficult to stain by gram method, since they give variable results (G+ve &G-ve), therefore Ziehl-Neelsen method (using carbol-fuchsin stain) is used to stain this bacterium**. **4-** When stained with **carbol-fuchsin by the Ziehl-Neelsen method**, **they resist de-colorization by 20% sulfuric acid and absolute alcohol for 10 minutes (acid and alcohol fast).** With this stain, the *Tubercle bacilli* are; 5- **bright red, while the tissue cells and other organisms are stained blue (as shown in the photos, below). 6-** Organisms in tissue and sputum smears often have a **beaded appearance because** of their vacuoles and **polyphosphate** content.

Acid fastness has been ascribed to the presence of **mycoloic acid in** the cell wall of bacilli. **It is related to the integrity of the cell and appears to be a property of the lipid-rich waxy cell wall**. Staining may be uniform or granular. In *M. tuberculosis* there are beaded forms frequently seen, but *M. bovis* stains more uniformly. *M. bovis* appear straighter, shorter with uniform staining.



***Mycobacterium tuberculosis* in Ziehl-Neelsen stained smear**

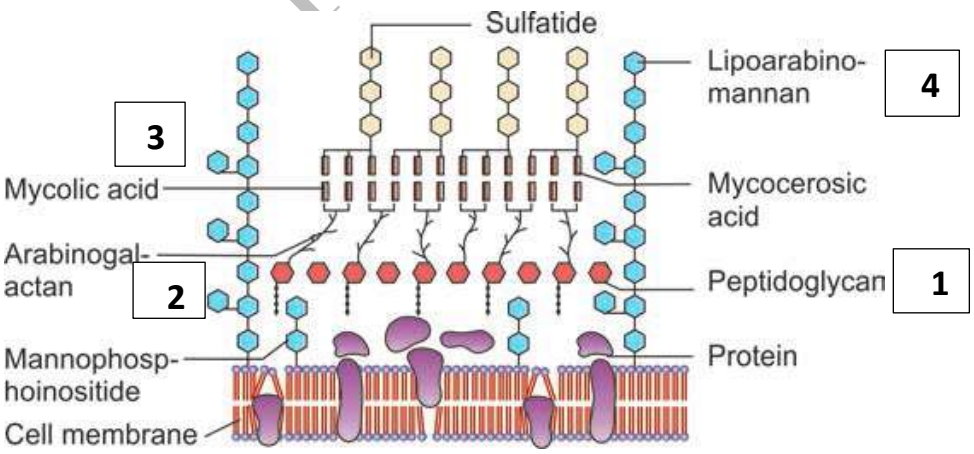
Cultural Characteristics: *M. tuberculosis* is **1- obligate aerobe.** The **optimal temperature** of tubercle bacilli is **37**°C. Optimum pH is 6.4 to **7**.0. **2- The bacilli grow slowly, the generation time *in vitro* being 14 to 15 hours. 3- Colonies appear in about 2 to 8 weeks. 4- The selective medium is Lowenstein-Jensen**

(LJ) medium without starch. 5- On LJ medium the colonies are <u>dry</u>, <u>rough</u>, <u>raised</u> and **<u>irregular</u> with a wrinkled surface**. **6-** They are **<u>creamy white, becoming yellowish or buff colored on further incubation.</u>**

Antigenic Structure

Mycobacteria possess two types of antigens, 1- the cell wall (insoluble) and 2- cytoplasmic antigens (soluble).

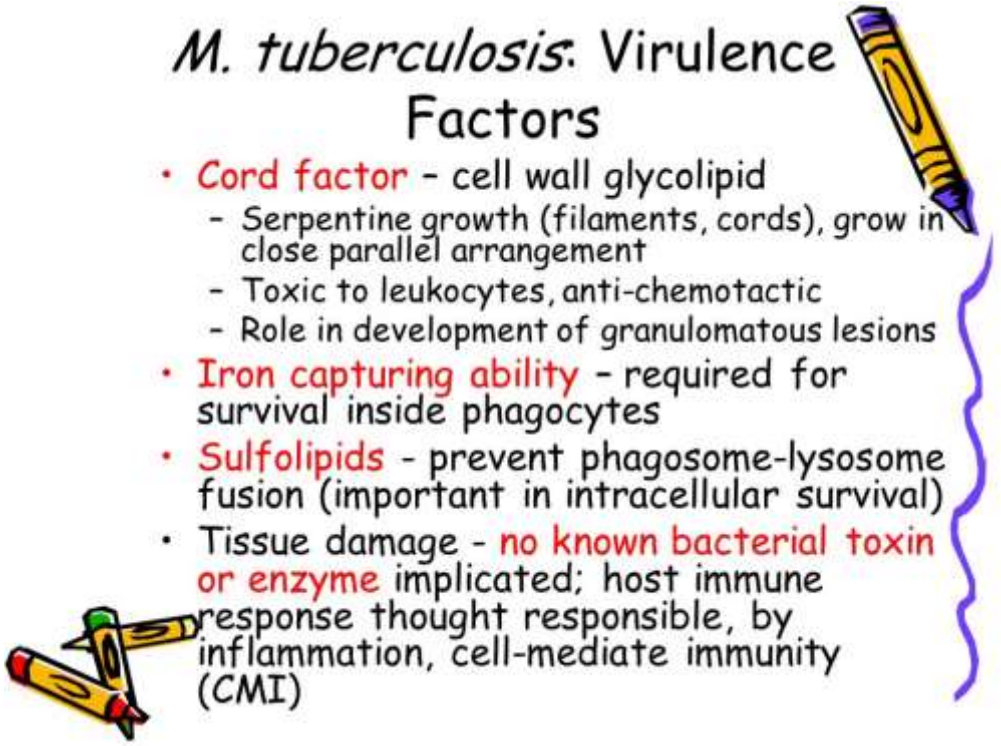
Cell wall antigens: The cell wall consists the following contents; 1) <u>lipids</u>, 2) <u>proteins</u> and 3) <u>polysaccharides</u>. T**<u>hese lipids constitute 60% of the cell wall weight and contributes to several biological properties.</u> Lipids of the cell wall (mycolic acid) are responsible for acid-fastness of bacteria and the cellular reaction of the body.** However, **the cell wall is made up of four distinct layers as the following: 1- Peptidoglycan layer** (murein). **2- Arabinogalactan layer**. **3- Mycolic acid layer. 4- lipoarabinomannan** (LAM).



<u>Cell wall of *Mycobacterium tuberculosis*</u>

Table 39.1 Comparison between M. tuberculosis and M. bovis

Test	M. tuberculosis	M. bovis
1. Morphology	Slender, long, curved	Short, stout, straight
2. Staining	Barred, beaded	Uniform
3. Growth	Eugonic	Dysgonic
4. Action of glycerol	Growth enhanced	Growth inhibited
5. Colony	Dry, rough, wrinkled, consistency, rough, tenacious difficult to emulsify, colour creamy white, buff coloured	Moist smooth flat consistency friable, white
6. Biochemical reaction		
(i) Niacin production	+	–
(ii) Nitrate reduction	+	–
7. Animal pathogenicity		
(i) Progressive disease in rabbit	– or mild lesion	+ (generalised lesion)
(ii) In guinea pig	+ (progressive, fatal disease)	+ (similar action)



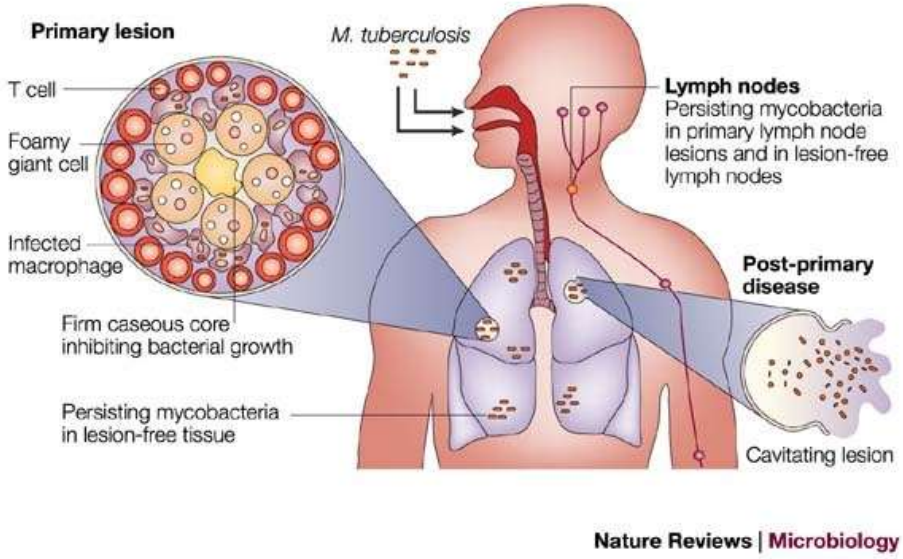
Specimen Collection: Persons who having pulmonary TB **should have at least <u>three</u> sputum specimens examined by smear and culture**. It is best to obtain a **series of early-morning specimens collected on three consecutive days**. Specimens should be obtained in an isolated, well-ventilated area.

For patients unable to cough up sputum, deep coughing may be induced by inhalation of an aerosol of warm, hypertonic (5%-15%) saline. Because induced sputum is very watery and resembles saliva, it should be labeled "induced" to ensure that the laboratory staff do not discard it.

Gastric aspiration can also be used to obtain specimens of swallowed sputum. During specimen collection, patients produce an aerosol that may be hazardous to health care workers or other patients in close proximity. For this reason,

precautionary measures for infection control must be followed during sputum induction, bronchoscopy, and other common diagnostic procedures.

Because TB can occur in almost any anatomical site, a variety of clinical specimens other than sputum (e.g., urine, cerebrospinal fluid-CSF, pleural fluid, pus, or biopsy specimens) may be submitted for examination when extra-pulmonary TB disease is suspected. Tissue specimens for the culture of *M. tuberculosis* should be placed in a transport medium (e.g., **Dubos**) or a normal saline solution.



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Prof. Dr. Habeeb Sahib Naher