Tuberculosis 2: Pathophysiology and microbiology of pulmonary tuberculosis

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Pathophysiology

Inhalation of *Mycobacterium tuberculosis* leads to one of four possible outcomes:

- Immediate clearance of the organism
- Latent infection
- The onset of active disease (primary disease)
- Active disease many years later (reactivation disease).

Among individuals with latent infection, and no underlying medical problems, reactivation disease occurs in 5 to 10 percent of cases [1]. The risk of reactivation is markedly increased in patients with HIV [2]. These outcomes are determined by the interplay of factors attributable to both the organism and the host.

Primary disease

Among the approximately 10 per cent of infected individuals who develop active disease, about half will do so within the first two to three years and are described as developing rapidly progressive or primary disease.

The tubercle bacilli establish infection in the lungs after they are carried in droplets small enough (5 to 10 microns) to reach the alveolar spaces. If the defense system of the host fails to eliminate the infection, the bacilli proliferate inside alveolar macrophages and eventually kill the cells. The infected macrophages produce cytokines and chemokines that attract other phagocytic cells, including monocytes, other alveolar macrophages and neutrophils, which eventually form a nodular granulomatous structure called the tubercle. If the bacterial replication is not controlled, the tubercle enlarges and the bacilli enter local draining lymph nodes. This leads to lymphadenopathy, a characteristic clinical manifestation of primary tuberculosis (TB). The lesion produced by the expansion of the tubercle into the lung parenchyma and lymph node involvement is called the Ghon complex. Bacteremia may accompany initial infection.

The bacilli continue to proliferate until an effective cell-mediated immune (CMI) response develops, usually two to six weeks after infection. Failure by the host to mount an effective CMI response and tissue repair leads to progressive destruction of the lung. Tumour necrosis factor (TNF)-alpha, reactive oxygen and nitrogen intermediates and the contents of cytotoxic cells (granzymes, perforin) may all contribute to the development of caseating necrosis that characterize a tuberculous lesion.

Unchecked bacterial growth may lead to haematogenous spread of bacilli to produce disseminated TB. Disseminated disease with lesions resembling millet seeds is termed miliary TB. Bacilli can also spread by erosion of the caseating lesions into the lung airways -and the host becomes infectious to others. In the absence of treatment, death ensues in 80 per cent of cases [3]. The remaining patients develop chronic disease or recover. Chronic disease is characterized by repeated episodes of healing by fibrotic changes around the lesions and tissue breakdown. Complete spontaneous eradication of the bacilli is rare.

Reactivation disease

Reactivation TB results from proliferation of a previously dormant bacterium seeded at the time of the primary infection. Among individuals with latent infection and no underlying medical problems, reactivation disease occurs in 5 to 10 per cent [1]. Immunosuppression is associated with reactivation TB, although it is not clear what specific host factors maintain the infection in a latent state and what triggers the latent infection to become overt. See previous article [4] for immunosuppressive conditions associated with reactivation TB. The disease process in reactivation TB tends to be localized (in contrast to primary disease): there is little regional lymph node involvement and less caseation. The lesion typically occurs at the lung apices, and disseminated disease is unusual unless the host is severely immunosuppressed. It is generally believed that successfully contained latent TB confers protection against subsequent TB exposure [5]

Microbiology

M.tuberculosis (MTB) belongs to the genus Mycobacterium that includes more than 80 other species. Tuberculosis (TB) is defined as a disease caused by members of the M. tuberculosis complex, which includes the tubercle bacillus (M. tuberculosis), *M. bovis, M. africanum, M. microti, M. canetti, M. caprae* and *M. pinnipedi* [6].

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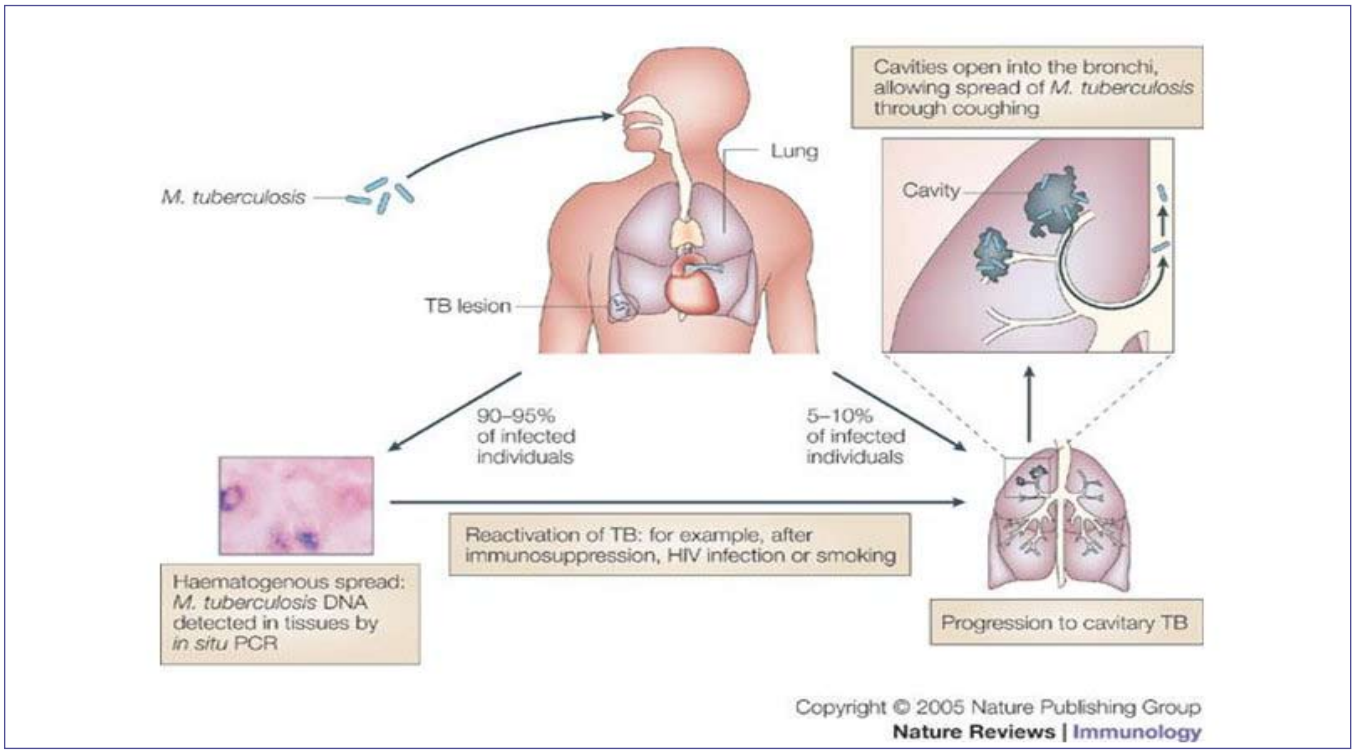

Figure 1. Pathophysiology of tuberculosis

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Cell envelope: The mycobacterial cell envelope is composed of a core of three macromolecules covalently linked to each other (peptidoglycan, arabinogalactan, and mycolic acids) and a lipopolysaccharide, lipoarabinomannan (LAM), which is thought to be anchored to the plasma membrane [7].

Staining characteristics: The cell wall components give mycobacteria their characteristic staining properties. The organism stains positive with Gram's stain. The mycolic acid structure confers the ability to resist destaining by acid alcohol after being stained by certain aniline dyes, leading to the term acid fast bacillus (AFB). Microscopy to detect AFB (using Ziehl-Neelsen or Kinyoun stain) is the most commonly used procedure to diagnose TB; a specimen must contain at least 10 [5] colony forming units (CFU)/mL to yield a positive smear [8]. Microscopy of specimens stained with a fluorochrome dye (such as auramine O) provides an easier, more efficient and more sensitive alternative. However, microscopic detection of mycobacteria does not distinguish *M. tuberculosis* from non-tuberculous mycobacteria.

Growth characteristics: MTB are aerobes. Their reproduction is enhanced by the presence of 5-10% CO2 in the atmosphere. They are grown on culture media with high lipid content, e.g. Lowenstein-Jensen (LJ) medium. The generation time of TB is approximately 12-18 hours, so that cultures must be incubated for three to six weeks at 370C until proliferation becomes microscopically visible. [9] Broth-based culture systems to improve the speed and sensitivity of detection have been developed [10]. In AFB smear-positive specimens, the BACTEC system can detect *M. tuberculosis* in approximately eight days (compared to approximately 14 days for smear-negative specimens [11,12].

Drug sensitivity testing: Drug sensitivity testing is increasingly important with the emergence of increasingly more resistant *M. tuberculosis* isolates. In addition to the conventional methods to test *M. tuberculosis* drug sensitivity, methods that rely on automated systems and PCR-based tests have been developed [13,14]. The microscopic observation drug sensitivity (MODS) test is another liquid culture drug-sensitivity test based on observation of M. tuberculosis growth in liquid broth medium containing a test drug. In an evaluation of 3,760 sputa samples using MODS, automated MB/BacT system, and Löwenstein-Jensen culture, sensitivity was 98, 89, and 84 percent respectively and the median time to the test results was 7, 22, and 68 days respectively [15]. The Xpert MTB/RIF is an integrated system that combines sample preparation in a modular cartridge system and real-time PCR. In 2010

this technique was recommended by the WHO to be used in place of traditional smear microscopy for the diagnosis of drug-resistant TB or TB in HIV-infected patients [16]. This test has been shown to have a sensitivity of greater than 98 per cent in sputum smear-positive TB cases and 75 to 90 per cent in smear-negative TB cases. The sensitivity in the detection of rifampicin resistant MTB exceeded 97 per cent, while specificity ranged 98 to 100 per cent. The test can yield results in less than two hour [17-19]. Here rifampicin resistance is assessed as a surrogate for multidrug resistant MTB.

Conclusion

South Sudan faces huge challenges in controlling tuberculosis. This is partly due to a limited laboratory network and lack of a tuberculosis reference laboratory (author's observation).

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