24 *Mycobacterium tuberculosis*

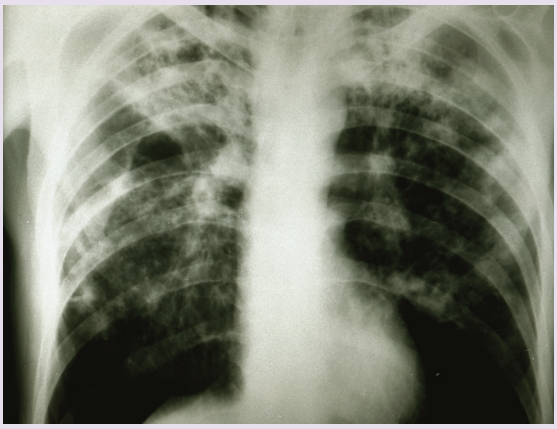

Figure 24.1 Chest X-ray of patient showing typical apical consolidation with possible cavities. There is also some consolidation adjacent to the mediastinum on the right. The bases are relatively spared. The medial aspect of the right hemi-diaphragm is just visible.

A 63-year-old man lived in a hostel for the homeless and sold magazines outside a railway station. He had been finding it difficult to cope with this recently, as he had been feeling weak, had lost weight, and often had a fever at night.

One month previously, he started coughing up blood and feeling breathless, which had really worried him. He was not registered with a primary healthcare provider but a friend told him about a walk-in practice for homeless people. Next day, he went to the practice and was seen by the physician on duty, who found that the patient had a low-grade fever and detected bronchial breathing when he listened to his chest. The doctor sent him for a chest X-ray and asked him to return for the results. When the X-ray result came back, it showed that he had apical shadowing and large **cavitation** consistent with tuberculosis (TB).

The X-ray is shown in **Figure 24.1**. A sputum sample was taken since the doctor suspected that the patient had TB and the patient was started an anti- tuberculosis therapy.

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

CAUSATIVE AGENT

This patient is infected with *Mycobacterium tuberculosis*. The stain used to identify mycobacteria is called the Ziehl-Neelsen (ZN) stain and characteristically stains mycobacteria red while all other organisms stain green (**Figure 24.2**). Culture and molecular tests are also used to identify Mtb and its antibiotic resistance (see diagnosis).

In common with Gram-positive bacteria, the cell wall contains a thick layer of peptidoglycan. Overlying this is a layer of arabinogalactan, which is covalently linked to the outer layer composed of mycolic acid, long-chain fatty acids specific for the mycobacterial genus, with other components such as glycophospholipids and trehalose dimycolate (also called cord factor as on staining the organism, it has the appearance of cords). Running vertically through the whole of the cell wall and linked to the cytoplasmic membrane is lipoarabinomannan – LAM (**Figure 24.3**).

The genus *Mycobacterium* can broadly be divided into rapid and slow growers and non-cultivable species. *Mycobacterium leprae*, the causative agent of leprosy and a close relative of the tubercle bacillus, cannot be grown on artificial media and can only be propagated in armadillos. Rapid-growing mycobacteria produce colonies within 2–3 days, for example *M. smegmatis*, while slow growers take more than 7 days. *M. tuberculosis*, a slow grower, can take 2–4 weeks to produce

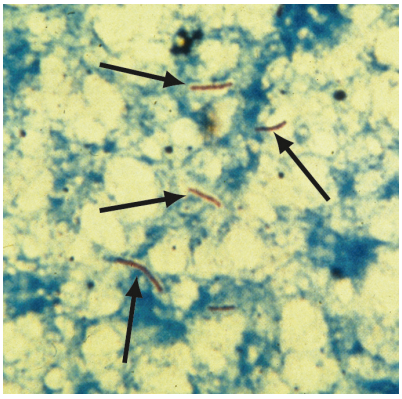

Figure 24.2 Ziehl-Neelsen stain of sputum: note the red bacilli (arrowed) against a green background.

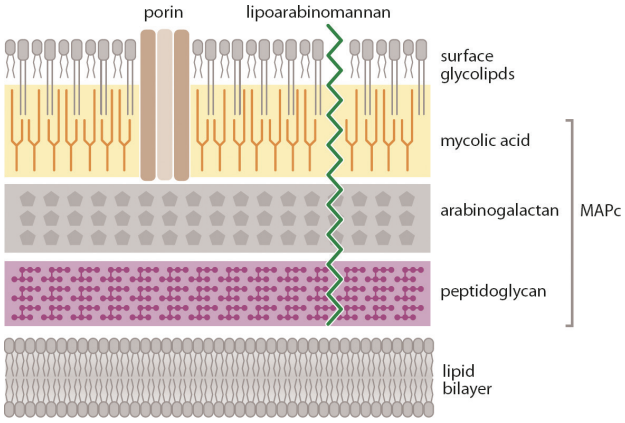

Figure 24.3 Model of the structure of the cell wall of mycobacteria. Note the mycolate-arabinogalactan-peptidoglycan-complex (MAPc). The mycobacterial cell wall is highly hydrophobic. The mycolic acid layer is impervious to many substances necessitating the presence of porin channels to allow entry of hydrophilic compounds. This layer plays a major role in the defence of the cell because few antibodies can penetrate it and it is relatively resistant to desiccation and some disinfectants.

colonies. This means that clinical decisions affecting treatment of TB and leprosy and the diagnosis of these conditions do not now rely primarily on culture. The slow growth also raises problems in determining the actual species causing illness (as the treatment may vary depending on the causative agent) and in determining antibiotic resistance. The usual medium for the isolation and growth of mycobacteria is Lowenstein-Jensen, which contains egg yolk and a dye (malachite green) that inhibits growth of more rapidly growing bacteria (**Figure 24.4**). There are also newer and faster methods of diagnosis used (see Section 4).

ENTRY AND SPREAD WITHIN THE BODY

Healthy individuals can be infected via the respiratory tract, the digestive tract, damaged skin, and mucous membranes. In primary infection, *M. tuberculosis* usually enters the respiratory tract in aerosol droplets, with 1–5 bacilli probably sufficient to cause infection. However, first they have to "run the gauntlet" while passing down the bronchus and bronchioles where mucosal epithelium produces anti-bacterial peptides (see Section 2). Those that make it to the alveoli interact with and enter alveolar macrophages. Entry into alveolar macrophages is mediated through a variety of surface receptors expressed by these phagocytic cells. Although the organism can multiply extracellularly to some extent within the alveolus, the organism is able to survive and multiply within the macrophages (due to mechanisms that prevent killing within the **phagosome** – see below). At this stage, macrophages die by programmed cell death (**apoptosis**) and release the mycobacteria. **Dendritic cells** also take up mycobacteria and become activated, which induces their migration to draining lymph nodes where they prime/activate T cells. Activated T cells recognizing mycobacterial antigens migrate into the lung and induce formation of small **granulomas** (see Section 2).

The site of infection in the lungs tends to be at the lung apex and close to the pleura. After a maximum of 3 weeks, and usually before cell-mediated immunity develops to any great extent, the microorganisms are released from macrophages and spread via the bloodstream to draining regional lymph nodes such as hilar or mediastinal. In addition, the bacilli spread to every organ in the body principally the lung apices, **meninges**, kidneys, and bones. Macrophages can also carry viable microorganisms around the body and how much of the spread occurs via this mechanism is unclear. Spread via the pulmonary arteries can give rise to **miliary** TB of the lungs which is, however, primarily found in immunocompromised patients and small children. Miliary TB is so named because the many tiny foci that form in the lungs resemble millet seed. Entry of the organism into the body via the mouth can cause laryngeal TB or intestinal TB.

It should be emphasized that primary infection with *M. tuberculosis* leads to **active** disease in only a small number of individuals (5–10%). Thus, most individuals are able to control the initial infection, showing either no symptoms or mild clinical manifestations similar to those seen for a common cold. However, most infected individuals carry the organism in a **latent** state for life under the control of an effective immune system (see below) although Mtb has many ways of avoiding the immune system (see Section 2). In the latent state, they are unable to transmit the infection but some individuals, estimated to be 10% or so in the US, develop active disease (reactivation) many years after primary infection. Reactivation TB often occurs when persons become immunosuppressed through HIV infection, diabetes or other conditions affecting the immune system. It is estimated that 20% of the world population have latent TB infection (LTBI) although the percentage varies from country to country.

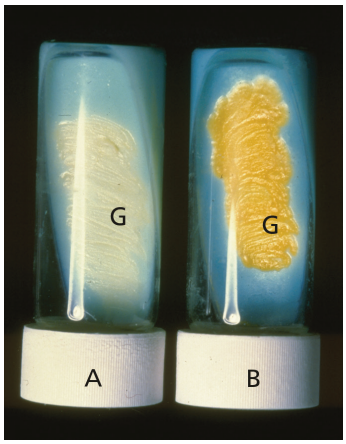

Figure 24.4 Growth of mycobacteria (G) on Lowenstein Jensen medium. The growth of *M. tuberculosis* does not produce a pigment (A) but the growth of another mycobacterial species produces a yellow pigment (B).

PERSON-TO-PERSON SPREAD

In patients with active TB, *M. tuberculosis* bacilli are released from granulomas into the bronchi and are spread through coughing. The aerosols produced contain droplet nuclei and survive for quite long periods of time outside the body. It is estimated that each infected person infects, on average, 20 other individuals. Repeated contact with an infected individual, particularly in a closed environment, produces higher transmission rates than casual contact. Similarly, if the infected person is smear-positive (mycobacteria seen in the sputum by ZN stain), indicating that bacteria are present in large numbers in the airways they are much more contagious, that is 50% of contacts may become infected. Whereas, if the index case is smear-negative (i.e. mycobacteria not seen by ZN stain of sputum) but is culture-positive, then only about 5% of contacts will be infected. The number of times an index case coughs is also directly related to the transmission rate.

EPIDEMIOLOGY

TB has been a human pathogen for thousands of years and, in 2019, was the eighth of the top ten causes of death in low-income countries. It was the seventh in middle-income countries and did not figure in the top ten of high-income countries.

It is estimated that about one quarter of the world's population (2 billion) is infected with *M. tuberculosis* and 5–10% of them will develop disease in their lifetime.

A total of 10 million people fell ill and 1.4 million died because of TB in 2019 worldwide. There were 5.6 million men, 1.2 million women and 1.2 million children. Eight countries accounted for two thirds of the total new TB cases in 2019, with India leading the count, followed by Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa. The incidence of TB for the above-mentioned countries and several other countries for the purpose of comparison is shown in **Table 24.1**.

At-risk groups for development of active disease in the population include prison inmates, the homeless, alcoholics, intravenous (IV) drug users, and those who are suffering social deprivation.

The good news is that the global incidence of TB fell at about 2% per year and between 2015 and 2019, the cumulative reduction was 9%. However, multidrug-resistant TB (MDR-TB) has increased and remains a public health crisis and a health security threat (see Section 5).

M. TUBERCULOSIS AND CO-MORBIDITIES

HIV

People living with HIV are on average 18 (15–21) times more likely to develop active TB disease than people without HIV. Probably, this is due to the immunosuppressive properties of HIV. The average prevalence of HIV in patients testing positive for TB in 2019 in the top eight high-burden countries was 7.8%. In 2019, around 208 000 people died of HIV-associated TB.

SARS-CoV-2 (COVID-19)

There are little data available at this time, but it is predicted that the pandemic of COVID-19, which was made official in March 2020, will have an impact on both detection of *M. tuberculosis* and clinical outcome of TB patients infected with COVID-19.

Due to lockdowns in many countries, and reluctance of individuals to attend clinics and due, in part, to GPs not having face-to-face meetings but relying on virtual appointments, the detection of the disease is likely to have been reduced. There is already evidence from several high TB-burden countries of large reductions in the monthly number of people with TB being detected and officially reported in 2020, especially in India, Indonesia, the Philippines, Sierra Leone, and South

Table 24.1 Estimated incidence of TB in 2019

Country	Actual cases	Rate/100 000 population
India	2 640 000	193
Indonesia	845 000	312
China	833 000	58
Philippines	599 000	554
Pakistan	570 000	263
Nigeria	440 000	219
Bangladesh	361 000	221
South Africa	360 000	615
Vietnam	170 000	176
Ethiopia	157 000	140
Russian Federation	73 000	50
Congo	20 000	373
UK	5132	7.7
USA	8920	2.7

Data from WHO Global Tuberculosis Report – 2020 Licence: CC BY-NC-SA 3.0 IGO.

Africa. The longer TB cases remain undetected, the greater the mortality risks.

Since both TB and Covid-19 are respiratory diseases, co-infection is likely to produce poorer treatment outcomes. This is also likely to be exacerbated by patients becoming infected with Covid-19 in countries where there is a high frequency of co-infections with TB and HIV.

Other co-morbidities affecting the risk of TB are diabetes, smoking, chronic obstructive pulmonary disease (COPD), and alcohol abuse.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

INNATE IMMUNITY

On entry into the respiratory tract, the mycobacteria in small droplets are exposed to the epithelial cells in the trachea, bronchus and bronchioles that through pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), produce a variety of antibacterial substances, for example defensins. Mucus produced by goblet cells contains defensins, together with some antibodies (especially IgA), lysozyme and cytokines. Defensins can disrupt the mycobacterial envelope. The mucociliary escalator can eliminate around 90% of inhaled microbes. If mycobacteria make it to the lower respiratory tract (the alveoli) they are then confronted with alveolar macrophages. These have a variety of receptors including complement receptors, scavenger receptors, Fcγ receptors, and PRRs that allow them to sense the mycobacteria and induce pro-inflammatory cytokines. PRRs include the TLRs, 2, 4, and 9 as well as others. Thus, recognition by the macrophage PRRs of the microbial-associated molecular patterns (MAMPs) on the mycobacteria leads to macrophage activation resulting in the production of the cytokines TNFα and IL6, and the chemokine CXCL8. This initiates an inflammatory response, which induces the recruitment of macrophages/monocytes from the circulation. Other important outcomes of macrophage activation include up-regulation of numerous antimicrobial effectors (NADPH oxidase, which generates reactive oxygen species, and iNOS which generates reactive nitrogen species, NRAMP). TLRs therefore play a critical role in early responses to *M. tuberculosis*. In fact, TLR polymorphisms have been associated with increased susceptibility to tuberculosis among different populations. The alveolar secretion itself also contains antibodies and molecules of the innate immune system including antibacterial peptides including collectins and complement proteins that all play a role in antibacterial immunity. Normally, having entered macrophages through phagocytosis, bacteria are killed within the phagolysosome. However, mycobacteria can live and divide within macrophages. They do this by inhibiting maturation of their phagosomes to prevent fusion with lysosomes and phagolysosome formation. This is mediated by some of their cell-wall glycolipids such as the cord factor or trehalose dimycolate. Thus, they are not exposed to the bactericidal content of the lysosome. There are many different innate immune evasion mechanisms used by *M. tuberculosis* (see below). Damage to the phagosomes also leads to escape of the organisms into the cytosol. The host may also respond by inducing a mechanism called "autophagy, which is not only the recycling system of the host cell but also a mechanism to target intracellular microorganisms to lysosomes. Neutrophils are also attracted to activated alveolar macrophages and are thought to provide antimicrobial proteins. NK cells recognize *M. tuberculosis* organisms and are thought to control their growth through granulysin and perforin, in a contact-dependent manner and also by enhancing macrophage-mediated killing. Gamma/delta T cells (Tγδ) are also activated by mycobacterial components and, like NK cells, directly kill organisms and enhance intracellular killing by macrophages. Other cells are thought to be important in immune responses to *M. tuberculosis*. These include NKT cells, and invariant NKT cells (iNKT cells). In addition, innate lymphoid cells (ILCs1, 2, and 3) and lymphoid tissue inducer cells (LTi) are also involved in anti-*M. tuberculosis* immunity. From experimental animal models, ILC3s are believed to be important in early protection against *M. tuberculosis*.

ADAPTIVE IMMUNITY

An adaptive cell-mediated response does not occur until 2–3 weeks after infection following the migration of lung macrophages and dendritic cells containing whole, or pieces of *M. tuberculosis* to the draining pulmonary lymph nodes. The mycobacterial components are broken down by proteolytic enzymes to produce peptides that are then presented via class II human leukocyte antigens (HLA) to CD4+ T cells, which, in the presence of interleukin (IL)-12/IL-18 produced by macrophages and dendritic cells, will differentiate to Th-1 cells. These Th-1 cells produce the pro-inflammatory cytokines interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α). Together with IFN-γ and IL-1, mainly produced by the macrophages themselves, these cytokines further activate the bactericidal effectors of macrophages such as defensins (e.g. cathelicidin), nitric oxide (NO) production and autophagy induction. Th-17 cells are also induced through the cytokines IL-1β, IL-6, and IL-23 produced by dendritic cells, macrophages, osteoblasts, or brain microglial cells, either in local lesions or in draining lymph nodes. Reduced levels of CD4+ T cells in HIV infection has been linked to the higher mycobacterial load and increased extrapulmonary dissemination in TB infection.

During mycobacterial infection, cytotoxic CD8+ T cells are induced through peptide antigens presented to them by class I HLA. These T cells produce IFN-γ but are also able to kill cells infected with mycobacteria by perforin and granzymes. Granulysin, released by cytotoxic T cells can also kill mycobacteria.

Although controversial, there is accumulating evidence to suggest that antibodies play a role in immune protection. Thus, it has been reported that antibodies to *M. tuberculosis* increase their uptake through opsonization (enhancement) by macrophages and inhibit the intracellular survival of the microbes. From animal models, there is also some evidence for a role for particular subsets of B cells playing a part in immunity to *M. tuberculosis*.

IMMUNE ESCAPE MECHANISMS OF M. TUBERCULOSIS

There are a large number of escape mechanisms used by Mtb allowing the organism to lie latent in the host and these are summarized in **Table 24.2**.

The immune response to *M. tuberculosis* through activation of pro-inflammatory responses (Th-1 cells and M1 polarized macrophages) is usually more damaging (immunopathology) than the bacterial infection itself. Early on in the infection, when the adaptive system becomes activated, small granulomas are formed in the lungs.

Table 24.2 Summary of the immune escape mechanisms used by *M. tuberculosis*

Inhibition of maturation of phagolysosomes
Inhibition of acidification of phagolysosome
Inhibition of oxygen stress
Inhibition of function of reactive oxygen and reactive nitrogen intermediates
Inhibition of apoptosis (strongly virulent Mtb)
Inhibition of autophagy
Formation of granulomas (see below)

Granuloma Formation

In most cases, the organism will survive and persist within some macrophages due to the numerous immune escape strategies used by Mtb (see above). They do not proliferate but persistence leads to continuous activation of both CD4 and CD8+ T cells. The cytokines released by these T cells and macrophages lead to the development of granulomas termed "tubercles" which gave the disease its name. Granuloma formation is a way of limiting the spread of infection and tissue involvement (**Figure 24.5**) and is one mechanism of escape from the immune system. The inability to eliminate the organism completely results in the production of a connective tissue layer to "wall off" the organism from the rest of the body. This is the containment phase (**latent** infection with dormant mycobacteria) and is called LTBI (latent TB infection).

Histologically, a granuloma is a collection of activated macrophages called epithelioid cells and a center that frequently shows an area of tissue **necrosis**. In tuberculosis, the necrosis is characteristically "cheesy" and is called caseous necrosis. Sometimes, the macrophages fuse to form giant cells. Lymphocytes, particularly of the CD4 T-cell subset but also CD8+ T cells, are also present in the granulomas and actively produce cytokines. They are formed by macrophages containing mycobacteria, epithelioid cells, and multinucleate giant cells all surrounded by T cells, which are mainly CD4+. Fibroblasts are also found in the outer layers, together with some dendritic cells and B lymphocytes.

The persistence of mycobacterial antigens in live and dead mycobacteria means that the T cells are continuously activated and the granulomatous response in tuberculosis is considered to be a **type IV hypersensitivity** response. This is a classic form of chronic inflammation through persistence

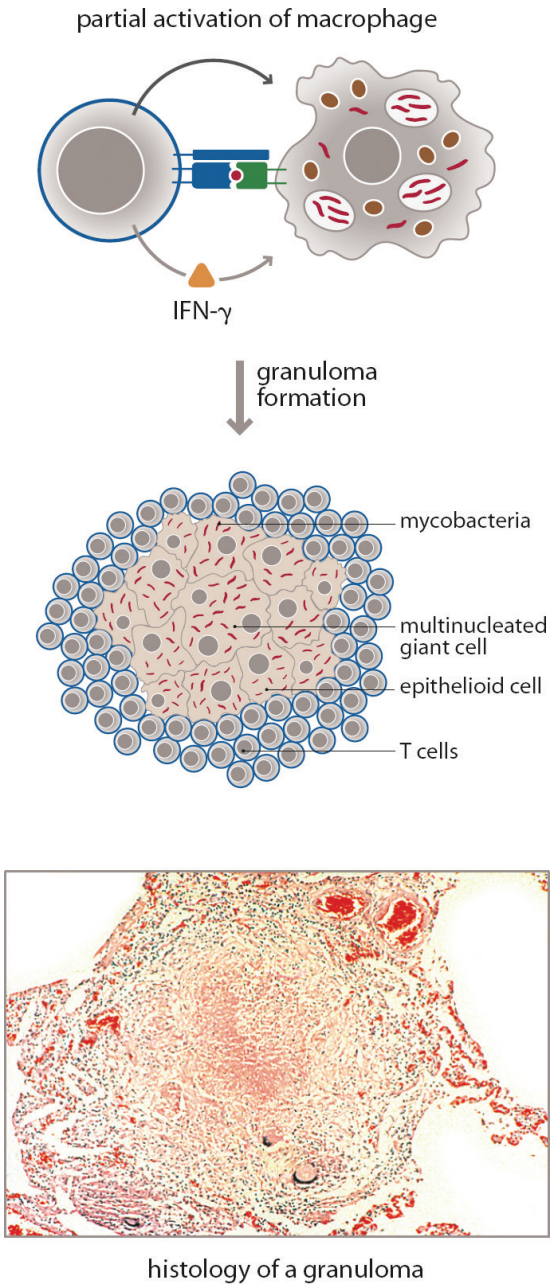

Figure 24.5 Granuloma showing macrophages containing mycobacteria, epithelioid cells, and multinucleate giant cells all surrounded by T cells, which are mainly CD4+.

of the infectious agent. However, M2 macrophages (anti-inflammatory macrophages) were found to predominate in both necrotic and non-necrotic granulomas from tuberculosis patients, while both M1 and M2 polarized macrophages were found in the non-granulomatous lung tissues. M1 macrophages promote granuloma formation and macrophage bactericidal activity *in vitro*, while M2 macrophages inhibit these effects. This suggests that M2 macrophages help to modulate the immune mechanism and are important in down-regulating the chronic inflammation resulting in an equilibrium between the immune system and the organism that keeps live organisms "in check".

At any time during life, the disease can be reactivated, leading to a change in status of these granulomas. Some of the granulomas cavitate (decay into a structureless mass of cellular debris), rupture, and spill thousands of viable, infectious bacilli into the airways (if they are in the lung). This leads to the person becoming infectious, as described above. Reactivation can also take place within granulomas at other sites in the body leading to active disease, for example, in the brain causing **meningitis**.

The host response depends on the immune conditions and dose of microorganisms.

1. Strong T-cell immunity and high dosage: there is greater tissue damage and **caseation**.
2. Strong T-cell immunity and low dosage: granulomas are produced.
3. Weak T-cell immunity (e.g. HIV co-infection): there is a poor granulomatous response, and many microorganisms are produced.

Figure 24.6 shows the possible sequence of events following infection.

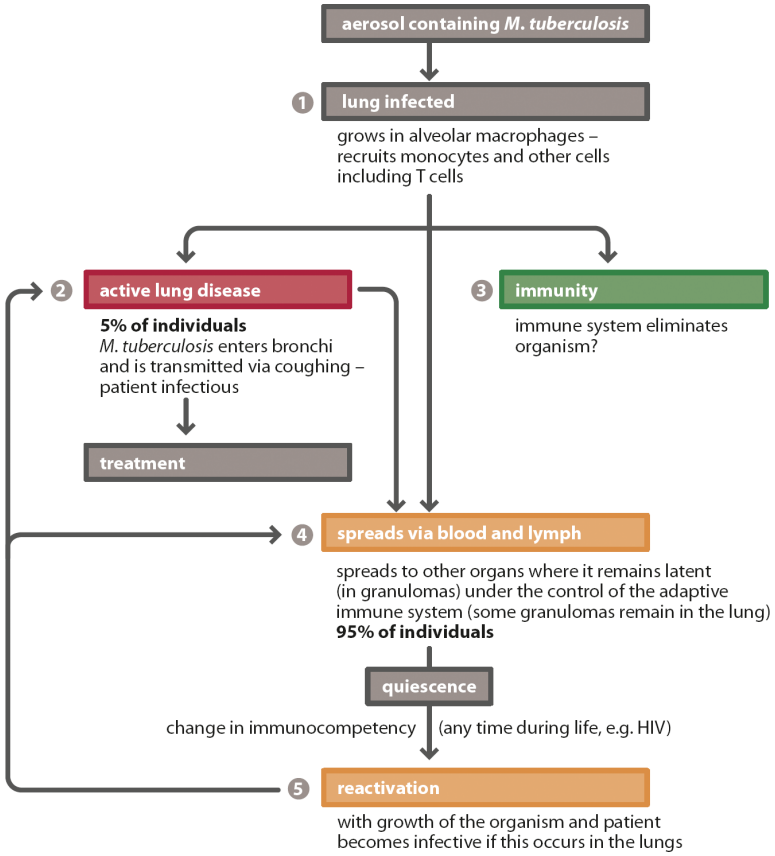

Figure 24.6 Sequence of possible events following tuberculosis infection. (1) In primary infections, *M. tuberculosis* organisms enter the lungs via aerosols and are taken up by alveolar macrophages and dendritic cells. The infection then moves to the lung parenchyma where monocytes and T cells are recruited through cytokines produced by the infected macrophages. The organism grows slowly within the resident and monocyte-derived macrophages to produce primary lesions or Ghon foci. These are small granulomas. Organisms from these foci can produce active disease in 5–35% of cases (2) and spread to other organs. In active disease, the foci become larger and result in significant tissue damage, release of the organism into the bronchi and transmission by aerosols (infectious stage). The patient develops symptoms at this stage and can infect other people. It is possible, but not proven, in some individuals that the organisms are totally eliminated from the body after primary infection (3). In primary infection, some microorganisms spread via the bloodstream and lymphatic system to other organs in the body where the immune system keeps the *M. tuberculosis* sequestered in granulomas (inactive) (4). At a later date, and probably as the result of a decrease in immunocompetence (e.g. HIV co-infection), reactivation of the growth of the *M. tuberculosis* within the granuloma occurs. This results in further spread and growth of the organism (5). Growth and active disease can develop in several organs other than the lung, including the pleura, brain (meningitis), lymph nodes, genitourinary tract, skin, joints, and bones. The rare infection of the spine (tuberculous spondylitis) is called Pott's Disease. If reactivation occurs in the lung, the individual becomes infectious.

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

The most common clinical presentation is fever, chronic productive cough that may be streaked with blood (hemoptysis), and weight loss. The release of T-cell and macrophage cytokines, particularly TNF-α, leads to fever (by its action on the thermoregulatory system of the hypothalamus) and weight loss. Adjacent granulomas in lymph nodes may fuse to produce a sizeable lump, which can be seen in a chest X-ray in the mediastinum or tissue destruction and cavities produced by dead tissue (cavitation, see **Figure 24.1**). The tissue destruction in the lungs resulting in cavitation can lead to loss of lung volume and erosion of bronchial arteries (cavitation, see **Figure 24.1**). This leads to hemoptysis. Spread of the organism through the body can lead to granulomas developing in other organs such as the brain, bone, liver, and so forth; perhaps the most common complication being the "space-occupying" effects of granulomas, for example, in the brain, where it can lead to **seizures**. Thus, TB can present with protean manifestations such as adrenal failure (**Addison's disease**) and fractures if it occurs in bone, for example, vertebral collapse (**Pott's disease**).

Several studies have been carried out that identify genes that associate with protection or predisposition to developing clinical TB, for example, alleles of HLADR (DRB1*1501 and 1502); alleles of NRAMP1 and alleles of IFN-γ. For more detailed information see Moller et al. (2018).

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

If pulmonary TB is suspected, then a chest X-ray is performed, and mycobacterial culture of sputum samples (**Figure 24.4**) is still considered the "gold standard" for TB diagnosis. The time to detection using culture for TB can be up to 2 months, significantly delaying the diagnosis of active TB in a large number of patients. However, culture methods that utilize both a liquid and a solid medium are now recommended and should allow detection within as little as 10–14 days. Although liquid systems are more sensitive and may increase the case yield by as much as 10%, they are also more prone to contamination. Although less sensitive, ZN-stained smear and microscopy is rapid and widely applied but it is only 50% sensitive at 5000–10 000 organisms per ml of specimen. However, Auramine which is a fluorescent stain can be used to rapidly scan a large area of a slide, resulting in a slightly higher sensitivity of 60%. Neither of these stains are specific for *M. tuberculosis* infection, they cannot differentiate between viable and dead bacteria, and are unable to detect drug-resistance. SybrGold is a DNA/RNA stain that has 90% sensitivity.

During the last 10 years or so, more rapid detection methods have been developed and endorsed by the WHO (consolidated guidelines on Tuberculosis, 2021). Nucleic acid amplification techniques (NAAT) using the **polymerase chain reaction** (**PCR**), are now used, usually in conjunction with culture. NAAT amplify specific sequences of the genome of *M. tuberculosis*, thereby detecting its presence in the specimen and, if used with liquid culture, can speed up the detection of the organism greatly. This method is both highly specific and sensitive and is particularly useful in detecting the presence of multidrug-resistant TB, as drug resistance is correlated with characteristic mutations in specific genes that can be detected by PCR. Using this test, Mtb can be detected in less than 2 hours outside the conventional laboratory and requiring only minimal healthcare skills. NAAT can identify mutations in the *rpoB* gene which codes for resistance to rifamycin, one of the first-line drugs used to treat TB and because this often coincides with isoniazid resistance, can serve as a surrogate marker for MDR (see treatments). A second-generation NAAT platform now enables detection of second-line drug resistance, i.e. fluoroquinolone and aminoglycosides/capreomycin.

In some laboratories, whole genome sequencing (WGS) is used for identifying specific mutations that lead to antibiotic resistance. Some of the common genes with mutations are: for isoniazid – *KatG*, *inhA*, *ahpC*; for rifampicin – *rpoB*; pyrazinamide – *pncA*, *rpsA*; ethambutol – *embB*; aminoglycosides – *rrs*, *tlyA*; fluroquinolones – *GyrA*, *GyrB*; *PAS* – *thyA*, *folC*.

An alternative diagnostic available outside the US utilizes unprocessed urine to detect the LAM antigen (lipoarabinomannan) an outer mycobacterial cell wall component that is shed into, and cleared by, the kidney (see **Figure 24.3**). However, the currently available urinary LAM assays have suboptimal sensitivity and specificity and are therefore not suitable as diagnostic tests for TB in all populations. Also, unlike traditional diagnostic methods, urinary LAM assays demonstrate improved sensitivity for the diagnosis of TB among individuals co-infected with HIV.

Blood diagnostic TB tests are available in some laboratories, such as the assay for IFN-γ from T lymphocytes activated by mycobacterial antigen (IGRA-interferon gamma release assay) and an **Elispot test** assaying the T-cell responses to mycobacterial antigens e.g. early secretory antigen target-6, (ESAT 6).

Diagnosis of Latent Tuberculosis

Transcriptosome signatures are able to distinguish between patients with active TB and TBLI and those patients with LTBI who go on to active diseases. A blood diagnostic test can also be used to test for LTBI.

The Tuberculin Skin Test (TST)

A small amount of tuberculin, also known as purified protein derivative, is a combination of proteins from Mtb that is

injected into the skin on the lower part of the arm (also called the Mantoux tuberculin skin test). A positive reaction for TB is indicated by a raised hard area or swelling as the result of a delayed-type hypersensitivity reaction after 48 to 72 hours. This indicates that the patient has been exposed to *M. tuberculosis* bacilli previously and could have LTBI but they will also require further testing for active disease.

DIFFERENTIAL DIAGNOSIS

It is important to note that the typical symptoms of weight loss, chronic cough, and fever may also occur in patients with tumors of the lung, for example, adenocarcinoma, squamous cell carcinoma, and oat cell carcinoma. Because *M. tuberculosis* spreads throughout the body and can present with signs and symptoms referrable to many systems, the infection can mimic many other diseases (e.g. brucellosis, lymphoma, glioma, meningitis).

Histologically, there are many other causes of granuloma that are infectious, for example, *Histoplasma* and *Bartonella* or non-infectious, for example berylliosis and silicosis and, in some cases, the antigen(s) is (are) unknown, for example sarcoidosis.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

MANAGEMENT

Infected patients in hospital should be isolated in a negative-pressure room where the air pressure outside the room is greater than that in the room. Thus, any airflow is into the room. Healthcare workers, such as physiotherapists, should wear close-fitting masks if they are involved in activities likely to induce coughing/expectoration by the patient. Once the patient has been on adequate treatment for 2 weeks, they can leave the isolation room.

The standard anti-TB regimen in the UK is a combination of isoniazid (with pyridoxine), rifampicin, pyrazinamide, and ethambutol for 2 months then isoniazid (with pyridoxine) and rifampicin for a further 4 months. This length of treatment, especially since patients may feel better before the end of treatment, may lead to lack of compliance.

A 4-month treatment for TB in which rifampicin and ethambutol substitute for rifapentine and moxifloxacin has been shown to have an equivalent success rate to the 6-month regimen.

TB is a notifiable disease in the UK. The contacts of patients with active TB should be screened by a chest X-ray or given prophylaxis, if appropriate. Close contacts of children with primary TB should be screened as there is likely to be a source that should be identified. Failure of therapy either due to inappropriate treatment or lack of compliance is important in development of drug-resistant strains. In the community, this can be controlled by giving directly observed therapy (DOT). This requires a healthcare infrastructure and appropriate finances.

TREATMENT OF LATENT TB

More than 80% of people who contract active TB disease in the US each year already have untreated LTBI. Treatment involves 1) isoniazid with rifapentine once weekly for three months, or 2) isoniazid with rifampin daily for three months. In addition, the WHO also recommend isoniazid daily for six or nine months as a primary regimen, whereas the CDC (USA) now lists these as alternative rather than preferred regimens. In contrast, rifampin daily for four months is the preferred regimen for the CDC (USA), but the WHO lists this as an alternative. The WHO guidelines also include two other regimens not included in the CDC guidelines: 1) isoniazid with rifapentine daily for one month, as an alternative regimen; and 2) for HIV-infected individuals in high TB transmission settings, isoniazid daily for 36 months.

MULTIDRUG-RESISTANT TB

Anti-TB medicines have been used for many decades and drug resistance emerges when anti-TB medicines are used inappropriately, through incorrect prescription by healthcare providers, poor quality drugs, and patients stopping treatment prematurely. TB strains resistant to one or more of the anti-TB drugs have been described in every country surveyed. In 2019, there was a global total of 206 030 people with multidrug (MDR) – or rifampicin-resistant (RR) TB (MDR/RR-TB), a 10% increase from 186 883 in 2018. About half of the global burden of MDR-TB is in three countries – India, China and the Russian Federation. There were 1.4 million incident cases of isoniazid-resistant TB, of which 1.1 million were susceptible to rifampicin.

MDR-TB is usually treatable and curable by using second-line drugs. However, second-line treatment options are limited, and require extensive drug treatment (up to 2 years of treatment) that involves expensive toxic drugs. In 2020, the WHO recommended a new shorter (9–11 months) fully-oral regimen for patients with MDB-TB. Research has shown that patients find it easier to complete the regimen, compared with the longer regimens that last up to 20 months.

Patients thought to have drug-resistant TB may be started on the above three drugs plus ethambutol until the actual sensitivity of the isolate is determined, when an appropriate combination of drugs can be given. Examples of second-line drugs include levofloxacin, moxifloxacin, bedaquiline, delamanid, linezolid, and pretomanid.

PREVENTION

Vaccination: BCG (Bacillus Calmette-Guerin) vaccine is an attenuated form of *M. bovis* (which causes TB in cattle) grown for many years on artificial medium. This is believed to provide variable protection against *M. tuberculosis* depending on the geographic location. It is generally 70–80% effective against the most severe forms of TB, such as TB meningitis, but less effective in preventing pulmonary TB. In most European countries, Canada, and the US, vaccination was abolished

some years ago, due to their low TB-transmission status and potential interference of vaccination with skin-test diagnosis. In the UK, US, and some other countries, BCG vaccination is now only given to specific high-risk populations. Such populations include: healthcare workers (see European policies for TB prevention in healthcare workers in references), laboratory staff, military personnel, and children in high-risk communities and countries. In high-risk countries, BCG is given to neonates.

A number of new TB vaccines are being developed that include various platforms including whole-cell vaccines, adjuvanted proteins, and recombinant subunit vector vaccines. One candidate vaccine (M72/AS01E) proved to be significantly protective against TB disease in a Phase IIb trial conducted in Kenya, South Africa, and Zambia, in individuals with evidence of LTBI. The point estimate of vaccine efficacy was 50% (90% CI, 12–71), over approximately three years of follow-up.

SUMMARY

1. WHAT IS THE CAUSATIVE AGENT, HOW DOES IT ENTER THE BODY, AND HOW DOES IT SPREAD A) WITHIN THE BODY AND B) FROM PERSON TO PERSON?

- *Mycobacterium tuberculosis* is an acid-fast bacillus, which grows very slowly.
- Because the cell wall is very impervious, few antibiotics can penetrate, and it is relatively resistant to desiccation and some disinfectants.
- The route of transmission is aerial, by inhalation of infected droplet nuclei.
- *M. tuberculosis* organisms grow in alveolar macrophages, can be released into the bronchi, and are spread through coughing.
- Active disease occurs in only 5% of individuals following primary infection.
- Most infected individuals carry the organism in a latent state for life, as the result of the many immune avoidance mechanisms used by *M. tuberculosis*.
- Some infected individuals may develop active disease many years after primary infection (5–10%) if they become immunosuppressed (reactivation).
- About 25% of the world's population (2 billion) is infected with *M. tuberculosis*, with around 10 million individuals developing active disease annually and 1.4 million dying in 2019.
- The main foci of infection are in India, Indonesia, China, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa.
- People living with HIV are 18 (15–21) times more likely to develop active TB.

2. WHAT IS THE HOST RESPONSE TO THE INFECTION AND WHAT IS THE DISEASE PATHOGENESIS?

- Both innate and adaptive immunity play a role in host defense.
- *M. tuberculosis* has many immune escape mechanisms including: inhibition of maturation and acidification of phagolysosome, inhibition of oxygen stress and function of both reactive oxygen and nitrogen intermediates, inhibition of apoptosis and autophagy. This allows the mycobacteria to live within the macrophage.
- Local tissue destruction is caused by the host response to the presence of mycobacterial antigens.
- Immune cells are activated, releasing cytokines, which cause further damage.
- CD4+ T cells are activated to produce cytokines to help with elimination of the *M. tuberculosis* organisms from the macrophages. Inability to get rid of all the microorganisms results in cytokine production leading to granuloma formation. This host response limits the spread of mycobacteria. A granuloma is a characteristic histologic feature of chronic inflammation. It is a collection of activated and resting macrophages called epithelioid cells surrounding an area of necrosis.
- Reactivation of the microorganisms within granulomas can occur at any time during later life through decreased immunocompetency (e.g. co-infection with HIV).

3. WHAT IS THE TYPICAL CLINICAL PRESENTATION AND WHAT COMPLICATIONS CAN OCCUR?

- Tuberculosis commonly presents with fever, weight loss, chronic cough, and there may be hemoptysis.
- Many sites other than the respiratory tract can be affected and it may present with signs and symptoms referrable to other organ systems.
- *M. tuberculosis* may cause seizures (CNS), meningitis (CNS), fractures (bones – Pott's disease) or Addison's disease (adrenal gland).

4. HOW IS THE DISEASE DIAGNOSED, AND WHAT IS THE DIFFERENTIAL DIAGNOSIS?

- Diagnosis of active tuberculosis is initially a clinical decision based on an X-ray (for pulmonary TB) and treatment is started on that basis.
- The simplest laboratory procedure is the Ziehl-Neelsen (ZN) stain but it is not absolutely specific.
- Specimens are cultured on Lowenstein-Jensen medium (which can take up to two months for diagnosis) and culture remains the gold standard for accurate diagnosis of tuberculosis although the bacterium can now be detected within 10–14 days.
- Newer nucleic acid amplification techniques (NAAT) using the polymerase chain reaction (PCR) are now used to detect Mtb present in the specimen which is also more rapid than culture and useful for detecting multidrug-resistant TB. WGS is used in some laboratories for identifying multidrug resistance genes and transcriptosome signatures can distinguish between TB and TBLI.
- Serologic tests detect the LAM antigen and blood diagnostic tests, e.g. IGRA are now available in some laboratories.

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- Tuberculin skin tests are used to test for latent TB.
- Tuberculosis may mimic tumors of the lung, brain, bone, intestine, blood, and other systemic granulomatous infections, for example, brucellosis.

5. HOW IS THE DISEASE MANAGED AND PREVENTED?

- The standard anti-TB regimen is a combination of rifampicin plus isoniazid plus pyrazinamide for 2 months, followed by rifampicin and isoniazid for a further 4 months, so the total treatment time is 6 months.
- Some regimens include ethambutol in the initial phase.
- MDR-TB has increased 10% since 2018 and about half of the global burden of MDR-TB is in India, China and the Russian Federation.
- A fully oral regimen for patients for second-line treatment of MDR-TB has been recommended by the WHO and second-line drugs include levofloxacin, moxifloxacin, bedaquiline, delamanid, linezolid, and pretomanid.
- TB is a notifiable disease in the UK.
- Effectiveness of BCG vaccination is highly variable between geographic areas. It is thought to be 70–80% effective against the most severe forms of TB such as TB meningitis but less effective against pulmonary TB. It is given only to "at-risk" groups including healthcare workers, children in high-risk communities and countries, military personnel and laboratory staff dealing with TB.
- A number of new TB vaccines are being developed that include various platforms including whole-cell vaccines, adjuvanted proteins, and recombinant subunit vector vaccines.

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Students can test their knowledge of this case study by visiting the Instructor and Student Resources: [www.routledge.com/cw/lydyard] where several multiple choice questions can be found.