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Heemskerk D, Caws M, Marais B, et al. Tuberculosis in Adults and Children. London: Springer; 2015.

Chapter 2 Pathogenesis

In this section the different phases of infection with *Mycobacterium tuberculosis* will be reviewed. Starting from transmission by inhalation, to the innate and adaptive immune response and the dual role of tuberculoma formation in walling off infection, but also providing an advantageous environment for bacilli to survive and multiply. Recent data has shown the role of Tumour Necrosis Factor alpha (TNF-α) in tuberculoma maintenance and its genetic control is more complex than previously thought. The role of vitamin D in susceptibility to tuberculosis also an area which has seen a resurgence of interest and new evidence emerging that targeted vitamin D therapy may have a role in improving TB outcomes.

2.1. Transmission

Transmission of TB is by inhalation of infectious droplet nuclei containing viable bacilli (aerosol spread). Mycobacteria-laden droplet nuclei are formed when a patient with active pulmonary TB coughs and can remain suspended in the air for several hours. Sneezing or singing may also expel bacilli. Factors influencing the chance of transmission include the bacillary load of the source case (sputum smear-positive or lung cavities on chest radiograph), as well as the proximity and duration of exposure (Escombe et al. 2008). Transmission is dramatically and rapidly reduced with effective treatment (Dharmadhikari et al. 2014). In general, the risk of infection among household contacts of TB patients is ~30 % (Singh et al. 2005) (Fig. 2.1).

For reasons not clearly understood, the majority of individuals infected with *M. tuberculosis* (~90 %) do not develop disease. Following inhalation of *M. tuberculosis* an individual may have one of the following outcomes: (1) fail to register an infection, (2) become infected but then clear the infection, (3) successfully contain the infection but continue to harbour bacilli in the absence of symptomatic disease (latent TB infection), or (4) develop progressive TB disease (Saenz et al. 2013). It has been estimated that one-third of the world population have latent TB infection and may be at risk to develop TB disease as they age, or become immunocompromised in the future. The factors resulting in reactivation of latent TB infection in the absence of overt immune suppression are not well understood, but the huge reservoir of individuals with latent TB infection represents a major barrier to TB elimination (Dye and Williams 2010).

Susceptibility to TB is influenced by environmental, host and pathogen factors. Innate immune responses play a crucial role in host defense against mycobacteria (Fig. 2.2). Although numerous gene polymorphisms have been identified which influence host susceptibility to TB, it is apparent that in the vast majority of cases susceptibility is polygenetic (Fitness et al. 2004). The complex interplay of multiple genetic variants has yet to be fully elucidated. On-going genome wide association studies (GWAS) studies should better illuminate genetic determinants of TB susceptibility and disease severity (O'Garra et al. 2013). In children immune maturation is a major determinant of risk with infants (<2 years of age) being at highest risk of disease development and potential dissemination (Perez-Velez and Marais 2012).

2.2. The Innate Immune Response

The key players in the innate defence against *M. tuberculosis* are the alveolar macrophages and dendritic cells. Macrophages, dendritic cells and other immune cells recognize mycobacterial structures, pathogen associated molecular patterns (PAMPs) with membrane associated pattern recognition receptors (PRRs), of which the most studied are the Toll-like receptors (TLR2, TLR4, TLR9). PAMPs such as lipoarabinomannan, phosphatidylinositol and heat shock proteins (Hsp65 and Hsp70), and mycobacterial nucleic acids, such as the CpG motif, are recognized by TLRs. On interaction with the TLRs, signalling pathways are activated which lead to the production of predominantly proinflammatory cytokines, such as TNF, IL-1B, IL-12 and nitric oxide (Kleinnijenhuis et al. 2009; van Crevel et al. 2002).

PRR-mediated phagocytosis of the pathogen by macrophages is an essential feature of the innate immune response. Ingested bacteria are then destroyed through phagosome-lysosome fusion and acidification (by H_2O_2 and other reactive oxygen intermediates) however *M. tuberculosis* may subvert this process and evade destruction (Sullivan et al. 2012). Essentially the innate immune response mediated through macrophages can have three major results; (1)

cell necrosis, (2) apoptosis (3) survival of the infected macrophages. If the cell undergoes necrosis, mycobacteria are released and may infect new macrophages or disseminate whereas an apoptotic cell membrane is not compromised and the bacteria are destroyed with the macrophage. Survival of infected macrophages enables the mycobacteria to persist and even proliferate before the adaptive immune response is activated by specific T-cells that have been selected in the regional lymph nodes; generally 2–3 weeks after primary infection (Saenz et al. 2013).

2.3. The Adaptive Immune Reponse

Dendritic cells are an important mediator between the innate and adaptive immune response which in addition to phagocytosis, present live mycobacteria to naïve T cells after migrating to regional lymph nodes. After antigen presentation in lymph nodes, CD4+ T cells are activated, and migrate to the lungs to impede mycobacterial progressive growth. The crucial role of T-cells in immunity to mycobacteria is evidenced by the dramatically increased susceptibility of individuals with HIV infection. Susceptibility to TB increases as the CD4 cell count decreases. IFN-γ, produced by activated T-cells, has a crucial role in protection against TB. IFN-knock-out mice, and humans with impaired IFN-γ genes are highly susceptible to severe TB disease (van Crevel et al. 2002). IFN-γ is essential in macrophage activation and intracellular mycobacterial killing (Flynn et al. 1993). TNF-α is another key cytokine produced by macrophages, dendritic cells and T cells and plays a central role in granuloma formation, macrophage induction and has immunoregulatory properties. Patients using TNF suppressing agent are at increased risk of infection and reactivation. A Cochrane review of TNF-α inhibitors given for any indication found a summary risk estimate odds ratio [OR] of 4.68, [95 %CI: 1.18–18.60] for reactivation of TB compared to control groups (Singh et al. 2011). However, TNF may also contribute to deleterious inflammatory responses in patients with progressive disease.

2.4. The Complex Role of TNF and Its Genetic Control

Although it is observed that TNF suppression can cause more rapid progression to TB disease, many aspects of the diverse functions of this proinflammatory factor have yet to be elucidated (Souto et al. 2014; Murdaca et al. 2015). Currently it is proposed that the effect of TNF on containment of mycobacterial infection is achieved by mediating the maintenance of granuloma integrity by regulating cell-adhesion proteins, chemokine attraction, and preventing T-cell dependent granuloma disintegration and inflammatory destruction by regulating IFN producing CD4+ and CD8+ T cells. A second mechanism is by promoting apoptosis of mycobacteria containing macrophages, rather than non-apoptotic death, thus preventing intercellular spread of bacteria (Miller and Ernst 2008).

It has been shown that in a Vietnamese population with TB meningitis that a polymorphism in the LTA4H gene which leads to either excessive or deficient TNF-α production can determine the response to adjunctive dexamethasone therapy. This polymorphism was initially identified in a zebrafish model of mycobacterial infection (Cronan and Tobin 2014). TB meningitis patients with an excessive TNF-α genotype appeared to benefit from adjunctive corticosteroids, with decreased mortality. While for those with a low TNF-genotype, steroids may actually be harmful, with increased mortality observed in this group when receiving steroids. It is possible that similar naturally occurring variants in the LTA4H genotype in all individuals exposed to TB may influence susceptibility and disease progression. It is now becoming apparent that rather than a simplistic model of high pro-inflammatory response being protective, the most protective response is balanced between pro-and anti-inflammatory mediators, or ‘just right’, which has led to the term ‘Goldilocks’ gene (Tobin et al. 2013).

2.5. The Tuberculoma

The hallmark of mycobacterial infection is the tuberculoma or granuloma. Our current knowledge on granuloma development in the human in the different stages of disease stems from meticulous post-mortem studies performed more than a century ago. Granulomas are described by gross pathological appearance: solid or non-necrotic, caseous or necrotic, or end-stage cavitary. Depending on the degree of liquefaction, the caseum (from Latin, cheese-like), can be referred to as liquid/soft or solid/hard. It is thought that in solid necrosis, the mycobacteria are more efficiently contained, and generally less viable mycobacterium are found in hard caseum. If sufficiently large, the granulomas may drain their (liquid) content into the bronchial tree, releasing viable bacilli into the airways, to be aspirated into other parts of the lung or coughed up and transmitted. If associated with parenchymal destruction it heralds the onset of lung cavities, where extra-cellular bacilli multiply exponentially. It has long been assumed that the granuloma formation serves the host in containing the bacilli and preventing bacterial spread but it may also be exploited by the bacilli to proliferate (Ramakrishnan 2012). Indeed many people have evidence of healed granulomas, without having

experienced active tuberculous disease. However, it is evident that control of infection within granulomas are not necessarily homogeneous within the same individual and ineffective in a substantial proportion of the global population.

On microscopic level the tuberculous granuloma is an organized aggregation of immune cells and debris. It contains macrophages that have undergone morphological change into epithelioid cells which form into zipper-like arrays around the necrotic centre. They retain the ability to phagocytise mycobacteria. Macrophages can also fuse to form multinucleated giant cells and foam cells, which have high lipid contents, but only few bacteria and their protective role is uncertain. Other cell types surrounding the granuloma are dendritic cells, neutrophils, B cells, T cells, natural killer (NK) cells, fibroblasts. Epithelial cells often are found in the outer layer of the granuloma. Mycobacteria are concentrated in the periphery of the central necrotic area.

2.6. Vitamin D and the Immune Response

In the pre-antibiotic era TB patients were often treated with cod-liver oil and sunshine, both sources of 25-hydroxyvitamin-D, which has immunomodulatory properties. Currently the interest in the role of vitamin D-status in susceptibility to TB and the use of vitamin D adjunctive to antimycobacterial treatment has been re-ignited (Nunn et al. 2013). Particularly in the context of multi drug resistance, adjunctive treatment with vitamin D may be of importance in TB patients as, second-line treatment regimens are less bactericidal and should be paired with an optimal immune response in order to effectively eliminate infection.

2.6.1. Vitamin D Metabolism

Vitamin D is historically associated with bone disease for its role in maintenance of calcium homeostasis by promoting calcium absorption in the intestines and bone resorption, processes which are regulated by parathyroid hormone. However, the anti-inflammatory properties of vitamin D are increasingly being investigated by researchers globally in the context of other conditions, such as diabetes, infectious and autoimmune diseases and cardiovascular disease (Theodoratou et al. 2014).

Dietary sources of vitamin D are limited, however fish liver oils and fatty fish naturally contain vitamin D. It is difficult however to get the acquired intake of vitamin D solely from natural dietary sources. Sunlight is a another source of Vitamin D, as after exposure to ultraviolet B, 7-dehydrocholesterol in the plasma membrane of human keratinocytes is converted to previtamin D3, from which vitamin D3 (cholecalciferol) is formed. Vitamin D is fat soluble and is carried in the circulation by hepatically produced vitamin D-binding protein. In the liver, vitamin D is hydroxylated to form 25-hydroxyvitamin (25(OH)D) (also known as calcidiol, the serum measure of vitamin D), which is converted to the steroid hormone 1,25-dihydroxyvitamin D (calcitriol, the biologically active metabolite) in the kidneys. The actions of the hormone are mediated either through ligation with a nuclear vitamin D-receptor (VDR) to regulate gene transcription, resulting in genomic responses, or via membrane rapid-response receptors (Ralph et al. 2013; Coussens et al. 2014).

2.6.2. Antimicrobial Effects of Vitamin D

Several mechanisms are proposed by which vitamin D may exert antimycobacterial properties and enhances the immune response. In particular the transcription of cathelecidin is completely dependant on sufficient levels of 1,25-hydroxyvitamin D (Aranow 2011). Cathelecidin destroys microbial membranes in the phagolysosome in macrophages.

2.6.3. Vitamin D Deficiency and Susceptibility to Tuberculosis

Vitamin D deficiency has been implicated to play a role in increased susceptibility to active TB disease in numerous studies.

25-hydroxyvitamin-D receptors are present on all immune cells, including macrophages, and are upregulated after stimulation of Toll-like receptors, which play a central role in mycobacterial recognition. Polymorphisms in the VDR receptor potentially modulate the activity of the receptor and thus the action of Vitamin D. A meta-analysis of the most widely studied polymorphisms in the VDR (*Fok*I, *Taq*I, *Apa*Iand *Bsm*I) and susceptibility to TB, showed that among Asians, the *Fok*Iff genotype had a positive association (OR 2.0, 95 %CI 1.3–3.2) with TB, whereas, a significant inverse association was observed for the *Bsm*I bb genotype (OR 0.5, 95 %CI 0.4–0.8). Marginal significant associations were found for *Taq*I and *Apa*I polymorphisms (Gao et al. 2010).

A meta analysis of 7 studies on vitamin D status and susceptibility to TB, including 531 individuals found that patients with tuberculosis have lower average pre-treatment serum levels of vitamin D than healthy controls matched on sex, age, ethnicity, diet and geographical location. The pooled effect size in random effects meta-analysis was 0.68 (95 %CI 0.43–0.93) (Nnoaham and Clarke 2008).

A systematic Cochrane review in 2011 found no consistent evidence of beneficial impact on TB treatment outcomes for micronutrient supplementation, including vitamin D (Sinclair et al. 2011). Supplementation of patients on treatment for pulmonary TB has been associated with accelerated resolution of inflammatory responses (Coussens et al. 2012). Supplementation led to accelerated clinical and radiological recovery as well as an enhanced immune response in those with deficient serum vitamin D at diagnosis in a recent trial (Salahuddin et al. 2013) (Fig. 2.3).

Figures

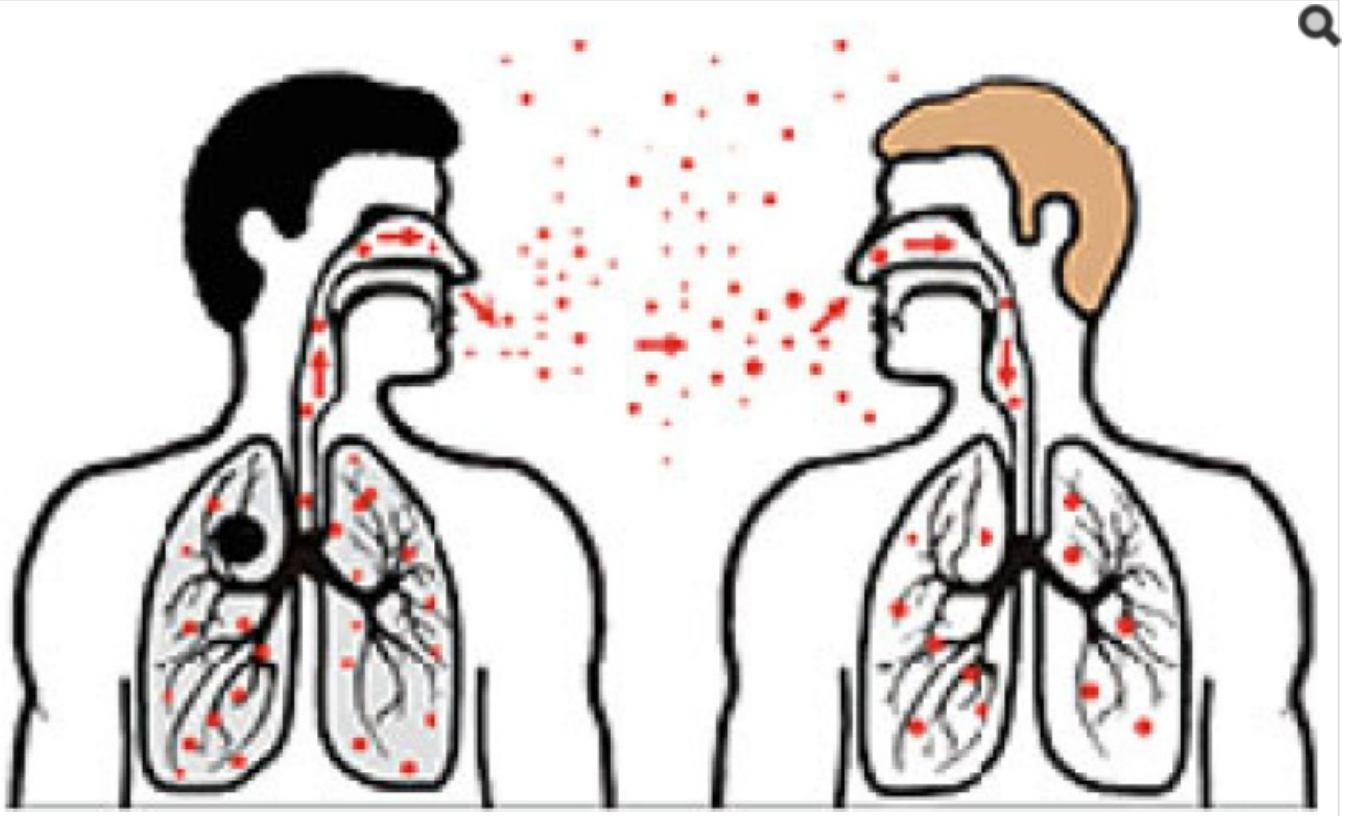

Fig. 2.1 Transmission of TB bacilli. *Source* CDC

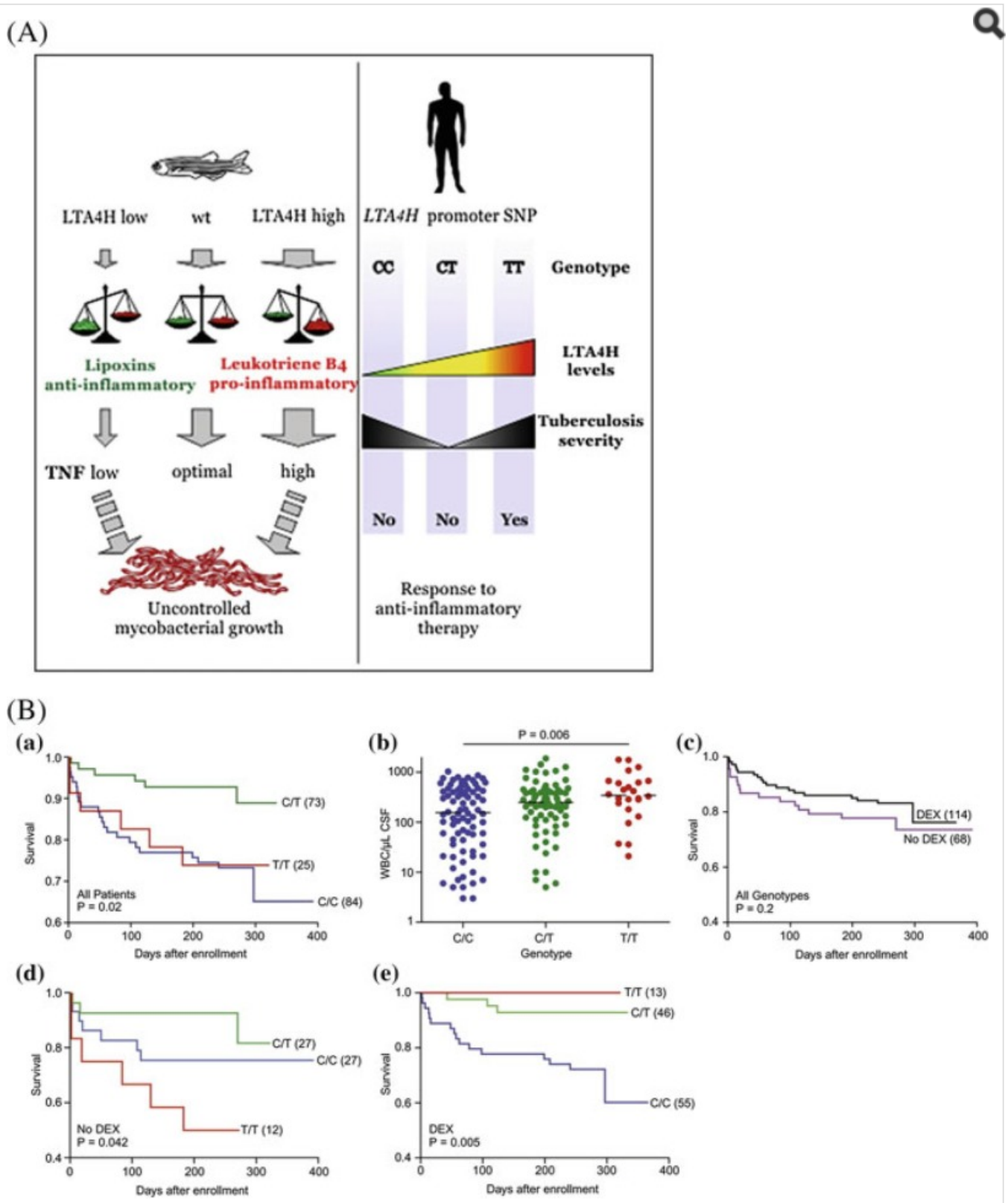

Fig. 2.2 Host genotype influences response to treatment with adjunctive steroids in Vietnamese patients with TB meningitis. A Humans may have polymorphisms in the LTA4H gene locus, which influence the severity of the inflammatory response. A process which is thought to be analogous to the susceptibility of zebrafish to *Mycobacterium marinum* infection. B Patients with the TT (high inflammatory) genotype, respond well to adjunctive treatment with dexamethasone. *From* Tobin et al., Cell, 2012, reprinted with permission

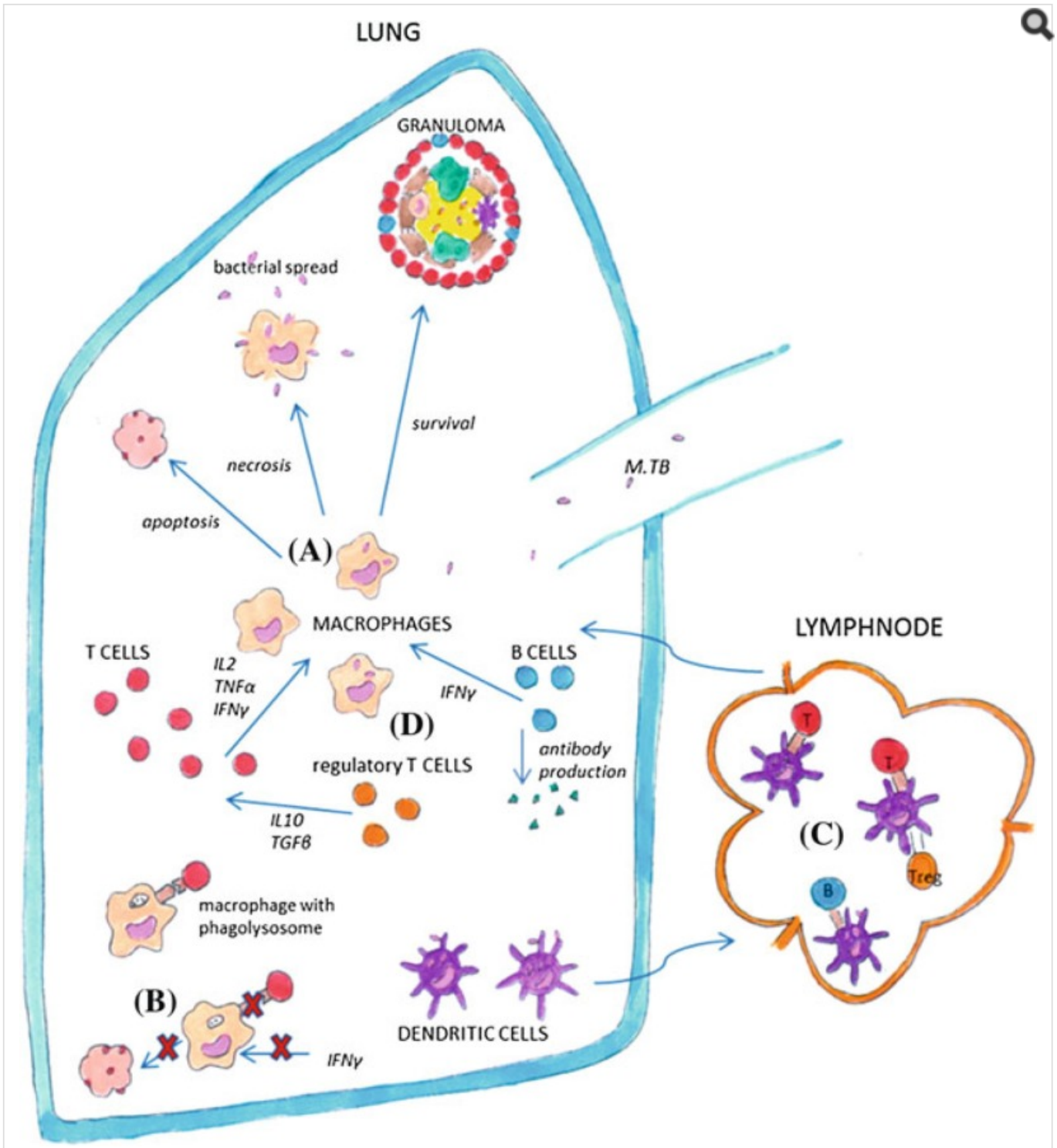

Fig. 2.3 Schematic representation of basic immunological antimycobacterial mechanisms in the lung and lymphnode. Macrophages and dendritic cells initially encounter Mycobacterium tuberculosis (M.TB) in the lung. *A* After ingestion, macrophages can undergo apoptosis or necrosis. After necrosis, bacterial spread may ensue. Surviving macrophages assist in early granuloma formation, either leading to elimination or clinical latency. *B* The mycobacteria can evade the immune response by inhibiting phagolysosome formation and apoptosis, as well as blocking the response of macrophages to IFNγ. *C* Resident dendritic cells of the lung can travel to regional lymphnodes, presenting live mycobacteria and mycobacterial antigen, activating naïve T-cells, B-cells and regulatory T-cells. *D* In the lung, activated T-cells and B-cells (attracted to the lung by chemokines) control bacterial growth by production of cytokines and antibodies. Regulatory T-cells control the inflammation through the production of IL-10 and TGF-β. Adapted from Saenz et al. Tuberculosis, 2013, adapted and reprinted with permission

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