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Lancet Infect Dis. Author manuscript; available in PMC 2021 June 01.

Published in final edited form as:

Lancet Infect Dis. 2020 June ; 20(6): e117–e128. doi:10.1016/S1473-3099(20)30148-1.

Cavitary Tuberculosis: The Gateway of Disease Transmission

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Summary:

Tuberculosis (TB) continues to be a major threat to global health. Cavitation is a dangerous consequence of pulmonary TB associated with poor outcomes, treatment relapse, higher transmission rates, and development of drug resistance. However, in the antibiotic era, cavities are often identified as the extreme outcome of treatment failure and are one of the least-studied aspects of TB. Here, we review the epidemiology, clinical features, and concurrent standards of care for individuals with cavitary TB. We also discuss recent developments in our understanding of TB cavities as dynamic physical and biochemical structures that interface the host response with a unique mycobacterial niche to drive TB-associated morbidity and transmission. Advances in preclinical models and noninvasive imaging can provide valuable insights into the drivers of cavitation. These insights will guide the development of specific pharmacological interventions to prevent cavitation and improve lung function for individuals with TB.

Introduction: The hole problem

Tuberculosis (TB) was responsible for an estimated 1·4 million deaths in 2018 and is among the leading causes of morbidity and mortality worldwide.[1] Cavitation is a seminal event and a key pathological feature of human TB. It has negative implications not only for the patient - associated with poor treatment outcomes, including delayed sputum culture conversion,

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Contributions

All authors contributed to the literature search. A.A.O, M.E.U, S.K.J, and W.R.B wrote and revised the manuscript. A.A.O, C.A.R-B, and M.E.U designed and created the figures. C.A.R-B conducted data retrieval and original analysis of CT scans in the TB Portals database. All authors reviewed and revised the final versions of the manuscript.

Declaration of interests

The authors declare no conflicts of interest.

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relapse after treatment, and development of drug resistance - but is also a public health threat, since cavitation greatly increases the risk of person-to-person transmission.[2,3]

A perfect storm of factors combines within the cavity to drive increased transmission, morbidity, and mortality (figure 1). In the most accepted model for TB cavity formation, the necrotic center of a granulomatous lung abscess erodes into an airway while some necrotic debris remains inside the newly formed cavity.[4] Phagocytes and granulocytes penetrate poorly into these necrotic areas creating an immune-sheltered zone of bacterial growth. High oxygen levels within the cavity also provide a rich environment with high rates of bacterial replication leading to a large bacillary burden at the inner edge of the cavity (10^7-10^9 bacilli), estimated to be a hundred thousand times higher than in necrotic TB lesions.[5–7] Rapid bacterial proliferation increases the frequency of replication-induced mutations and the likelihood of developing drug resistance.[8–14] These concentrated bacilli are poised to be expelled out of the lungs through the bronchial tree during transmission events. Finally, the inner contents of cavities are also poorly vascularized which limits the penetration of anti-mycobacterial drugs and may further promote selection for drug-resistant mutants.[15–18]

Apart from providing a growth niche, cavity airspace is also not useful for respiration. During cavity formation, both the basement membrane and alveolar architecture are permanently destroyed. Even after successful TB treatment, TB cavities can persist, leading to lifelong pulmonary deficits and recurrent opportunistic infections.[19]

In this review, we discuss recent developments in our understanding of TB cavities as dynamic physical and biochemical structures that interface the host response with a unique mycobacterial niche to drive TB-associated morbidity and transmission.

The clinical importance and epidemiology of TB cavities

Estimated rates of cavitary TB at the time of diagnosis range from 29 to 87%.[3,20–25] However, these rates could be overestimated as cavitary patients are more likely to have positive sputum samples, and thus easier to diagnose. Similarly, while chest radiography is a clinical standard in TB diagnostics, it may underestimate the presence of cavitation compared to computed tomography (CT) scans.[26] Rates of cavitation are higher in diabetic patients,[10,27] but lower with poorly managed HIV co-infection (although increased cavitation is seen after six months of antiretroviral therapy)[28], transplant recipients, and elderly patients.[29,30] Finally, the differences in risk of cavitation attributed to infection by different *M. tuberculosis* strains (e.g., Beijing genotype) are still unclear.[10,31–33]

Cavitary TB carries a poor prognosis. There is a higher risk of treatment failure and relapse if cavities are radiographically present during the first two months of therapy.[2] Then, if cavities persist after six months of treatment, the risk of relapse doubles compared to those whose cavities close by treatment completion.[34] The association between cavitation and relapse could be attributable to poor drug penetration into the poorly vascularized cavity. Alternatively, cavitation could be a marker for high bacillary burden from extensive disease.[2] Cavitary TB may also result in life-threatening sequelae (e.g., Rasmussen aneurysm).[35]

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Individuals with cavitary disease are also a risk to their community and contacts. Higher bacterial burdens were detected in sputum samples from cavitary TB patients,[3] and both the presence of cavities and their proximity to an airway correlated with increased coughing during anti-TB treatment.[36,37] Therefore, cavitary TB patients with higher bacterial burden are more likely to aerosolize and release *M. tuberculosis* leading to more transmission events.[38] Outbreak and case studies suggest that cavities are the likely pathophysiologic driver behind TB super-spreaders.[39,40] However, there is no consensus on the exact contribution of individuals with cavitary TB to the total volume of transmission events, and the need for selective isolation precautions based on individualized radiographic findings is still debated. One possible research avenue to address this knowledge gap is to build upon computational models of TB epidemiology using prevalence estimates for cavitary TB with stratified case-specific transmission risks to resolve the potential benefits of isolation precautions for patients with cavitary disease.[41]

The architecture of a cavity

A TB cavity is a pathologic gas-filled space in the lung parenchyma with a border, or wall, which was caused by infection with a pathogen of the *M. tuberculosis* complex.[42] Among TB cavities, there is heterogeneity in size, morphology, and wall-composition, which can be evaluated non-invasively by radiological images and by post-mortem analysis of gross appearance or histologic characteristics.

Imaging

Non-invasive anatomical imaging (e.g., X-ray and CT) allows cavities to be evaluated according to size (correlated with the extent of disease), shape, and wall thickness (figure 2A).[43] Despite common belief, radiographic imaging is unable to reliably determine the age of a cavity.[44–46] The radiological manifestation of TB cavities is heterogeneous, with some patients having single or multiple cavities, surrounded by a consolidation, fibronodular, or mixed pattern. Upper lobe cavities are commonly seen in immunocompetent adults, while cavities in the lower lobes associated with adenopathy and pleural effusions are commonly found in children and immunocompromised adults. Multiple adjoining small cavities can also fuse together to produce a large cavity.[26] Thicker cavity walls are associated with higher concentrations of bacilli in sputum, while thinner walls are usually observed after successful treatment.[43] Air-fluid levels are seen in 10%-20% of TB cavities, and endobronchial spread (small nodules distant to the cavity) is also evident in 10-20% of cases (figure S1).[47]

Most TB cavities occur in the apical or posterior segments of the superior lobes and, in smaller numbers, the upper segments of the inferior lobes. We analyzed the location and size of 287 cavities in CT scans of 143 patients with cavitary TB from the National Institutes of Health TB Portals Database. We found that 58% of all the cavities were localized in the apical segments, while 21% were located in the inferior lobes, with a distribution pattern similar to those previously reported (figure 2, figure S2, appendix).[48,49] While most large cavities occur in the lung apices, some can also be found in the upper segments of the inferior lobes, and smaller nodular cavities occur throughout the lungs (figure 2C).[49] Historically, this distribution toward ‘vulnerable regions’ at the apices of the lungs was

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attributed to reduced vascular supply, higher oxygen tension, and impaired lymphatic drainage in these regions compared to the inferior lobes.[50,51] However, the actual mechanism behind apically-oriented TB cavitation is still poorly understood.[52,53]

Gross appearance

The superficial surface of the lungs from individuals with pulmonary TB appears studded with areas of pneumonia that have a beige color (figure 2D, figure S3). Some areas appear as discrete and well-circumscribed nodules, the classic appearance of a proliferative lesion. Other areas are large and physically change the appearance and texture of an entire lung region or lobe and exemplify an exudative lesion. Some pneumonic nodules may contain cavities, but most cavities are usually not visually identifiable by looking at the surface of the lung.

When a cavity-containing lung lesion is dissected, an open space at the center identifies a cavity. Two distinct textures of wall material can often be identified as part of the wall. The inner surface of the cavity is often composed of caseum composed of bright-white material with a friable consistency similar to, or more liquid than, blue cheese, which can be easily removed from the cavity and is rich in cholesterol, cholesterol esters, triacylglycerols, and lactosylceramides (figure S4).[54] The remainder of the wall is located more peripheral to the cavity and usually has a dull color and stiff texture. This material cannot be easily removed and forms the stiff structure of the cavity wall.

Histologic patterns

The wall of a TB cavity typically contains three regions (figure 2E), though there is significant heterogeneity in the size and composition of these regions both within a single cavity and between different cavities. This heterogeneity may be related to many factors, including immune status, age of the cavity, size of the cavity, and anatomical location of the cavity.

First, at the boundary between normal-appearing lung tissue and the cavity wall begins a region of granulomatous pneumonia where the underlying alveoli, vasculature, and lung-tissue remains intact (GP_A), though filled with inflammatory cells including activated macrophages and clusters of lymphocytes that sometimes form a more defined lymphocytic cuff encircling the cavity. Organized tertiary lymphoid follicles, composed of antigen-presenting cells, $CD8^+$, $CD4^+$, and B lymphocytes, are also found within the GP_A and the surrounding lung tissue.[55] Second, moving towards the interior surface of the cavity wall is a region of granulomatous pneumonia where the alveolar structure and basement membrane are effaced (GP_E). Fibroblasts are present and participate in pathologic collagen remodeling. Greater amounts of fibrosis are commonly associated with older lesions. Intracellular mycobacteria are found sporadically within macrophages throughout the GP_E.[9] Third, along the interior surface of the cavity wall is a layer of necrotic cellular debris (NL), which is the microscopic identity of grossly visible caseum. The morphological type of necrosis can include elements of both caseous necrosis and liquefactive necrosis (figure S4). The border between the GP_E and the NL is marked by the depletion of extracellular fibrotic filaments leaving the NL devoid of basement member or fibrotic matrix. Finally, the NL typically

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contains high concentrations of extracellular mycobacteria and some mononuclear cells with lymphoid morphology.[9]

Experimental models of tuberculosis cavitation

Modeling cavities for preclinical studies is challenging since cavities are the consequence of complex and heterogeneous host-pathogen interactions (figure 3). Multiple animal models have been developed and each has advantages. The rate of cavitation exhibited by each model is an important parameter for experimental design and power calculations, but the variability in bacterial strains used, methods of infection, implantation doses, disease incubation periods, and readouts make comparisons between models difficult (table S1).

Non-human primate (NHP) models

M. tuberculosis-infected NHPs (cynomolgus and rhesus macaques, and common marmosets) demonstrate all of the major pathologic landmarks of TB including cavitation.[56] The reported incidence of cavitary TB in NHP models ranges from zero to 63% (table S1). However, few studies use NHPs as an exclusive model for cavitation. Via *et al.* showed that cavitary disease in common marmosets hindered bacterial clearance during chemotherapy, especially when a suboptimal drug combination was used for treatment.[57] This experimental study supports many population-based findings demonstrating cavitary disease as a risk factor for treatment failure and provides a tractable NHP model to further investigate the mechanistic drivers linking cavitation and treatment outcomes.

NHPs are an extensively validated pathologic and immunologic model for TB. Advantages include being amenable to high-resolution imaging studies and having the availability of many optimized immunological reagents.[58] However, the cost of experiments with NHPs could be a limiting factor where power calculations show that large numbers of animals are needed for experiments in which less than 50% of study animals develop cavities.

Rabbit models

Similar to NHPs, rabbits infected by aerosolized *M. tuberculosis* develop a spectrum of lesions similar to human disease, including cavities.[59] Early studies used trans-thoracic injections to identify the cavity-inducing chemical constituents of the *M. tuberculosis* bacillus.[60,61] Subsequently, Converse *et al.* characterized cavitation in rabbits following aerosol exposure to *M. bovis* and showed a correlation between infective dose and the frequency of cavitation.[62] However, reliable cavitation in the *M. tuberculosis* aerosol-infected rabbit model was scarce. Recently, Urbanowski *et al.* reported a variation on the rabbit aerosol model based on serial low-dose (~500 bacilli) aerosol exposures showing a higher incidence of cavitation (60-80%) compared with a single exposure (15-25%).[63] The results on this model support population studies identifying repetitive exposure as an epidemiological risk-factor for cavitation,[64] and provide an additional experimental tool for generating cavities in rabbits at higher rates.

Another well-established rabbit cavitary TB model uses bronchoscope instillation and generates cavities at targeted lung foci one month after infection in nearly 100% of the rabbits.[65] A salient difference between published models for bronchoscope instillation in

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rabbits versus NHPs is that a high-burden inoculum is employed in rabbits to generate cavities (10^4 bacilli) compared to general NHP infection models where many use < 30 bacilli (table S1).[66,67] Therefore, the infection and early events toward cavitation in the rabbit bronchial instillation model are highly artificial and may not be appropriate for studies on the natural pathogenesis of cavities. Nevertheless, cavities generated by bronchial instillation in rabbits have the major structural hallmarks of TB cavities and may be the best model for studying extensive apical cavitation and in pharmacokinetic studies.[68]

Overall, the rabbit model offers a robust, flexible, and cost-effective system for investigating cavitation. As with NHPs, rabbits are relatively outbred leading to heterogeneity in the disease response. The lungs of rabbits are also large enough for high-resolution imaging studies. However, the lack of immunological reagents is a major drawback to this model.

Mouse models

Until recently, mice had been overlooked as models of cavitary TB because laboratory mouse strains do not exhibit caseation or cavitation following infection with *M. tuberculosis*. Beginning in 1998, Kramnik *et al.* and others observed that the C3HeB/FeJ mouse strain developed necrotic and hypoxic inflammatory lesions that were more similar to human TB lesions than traditional mouse models.[69–71] Initial reports of occasional cavitation in C3HeB/FeJ mice led to further investigation of their potential use as a cavitary TB model. Ordonez *et al.* used this mouse strain to develop and characterize it as a model of cavitary TB.[72] Cavities were observed in 47-61% of the *M. tuberculosis*-infected mice (table S1), and displayed many of the histologic hallmarks of human cavities including matrix destruction, collagen remodeling, multinucleated giant cells, and an interior necrotic layer with a high extracellular bacillary concentration.[72]

Although recently characterized, mouse models of cavitary TB offer many advantages including genetic manipulation, a large variety of immunologic reagents, large numbers of cavities per experiment, and reduced costs over rabbits and NHPs. However, the large bacterial burden in the necrotic lesions of the C3HeB/FeJ mouse model of cavitary TB does not always represent the heterogeneity observed in human pathology, which can confound the analysis of some drivers of the cavitation process. Similarly, the small size of the lung may limit the utility of mice in studies focused on the biophysics of cavitation.

The pathogenic drivers of cavitation.

The process by which TB cavities form is still debated. Serial radiological observations suggest that TB cavities arise from pre-existing hyperdense lung regions that erode into adjacent airways.[4,26,63,72–74] This inference is supported by histological observations showing structural homology between the walls of cavities and necrotic granulomas. These studies also identify central necrosis and extracellular matrix (ECM) depletion as morphological changes required for cavitation.[63,72,74,75] However, an alternative and possibly concomitant cavitation process could arise from obstructive bronchopneumonia.[76]

Cavity formation is the conversion of immune accessible lung tissue to immune sheltered surfaces continuous with the external environment. We consider TB cavitation as a complex

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phenotype driven by biochemical, biophysical, immunological, and microbiological processes, which have important roles during the different stages of cavitation and therefore could be targeted for prevention of extensive lung destruction, disease transmission, and the emergence of drug-resistance.

Biochemical drivers

Proteolytic depletion of the ECM in the lung is necessary for TB cavitation, but the biochemical events leading to cavitation occur in two sequential steps. First, pathologic matrix remodeling occurs throughout pre-fibrotic granulomas and within the GP_E region of cavities when the alveolar tissue is effaced and replaced by fibroblast deposited fibrotic matrix. The loss of basement membrane causes irreversible damage to the lung and is probably driven by immune cells moving toward the center of the granuloma which secrete matrix-remodeling proteases to aid in migration. In the second step and focused at the boundary between the GP_E and the NL region, a dramatic depletion of extracellular fibrotic fibrils is tightly coupled with extensive necrosis. In the absence of an extracellular scaffold, either basement membrane or fibrosis, caseous necrotic debris can be evacuated with little mechanical resistance during the formation of a cavity.

Since the 1960s, when Dannenberg *et al.* reported enzymes capable of hydrolyzing proteins present in the necrotic lesions of *M. tuberculosis*-infected rabbits, multiple biochemical drivers of ECM destruction in cavitary TB have been described.[77–79] More recently, host-expressed extracellular collagenases, such as matrix metalloproteinases (MMPs) and cysteine cathepsins, have been described as key mediators of the ECM degradation that precedes cavitation in TB (figure 4A).[80–82] Higher expression of the MMP-1, 3, 7, 8, 9, 12, 13, and the cysteine cathepsin K have been described at the wall of TB cavities and granulomas.[54,65,72,81,83] Hypoxia augments monocyte and neutrophil MMP secretion acting through the hypoxia inducible factor (HIF)-1α transcription factor, an important regulator of the host response to oxygen deprivation.[84] Elevated concentrations of MMP-1, 2, 3, 8, and 9 have also been reported in respiratory fluids from pulmonary TB patients and correlate with severity of disease and number of cavities.[82,83,85,86] However, despite experimental and observational evidence linking extracellular collagenases to lung destruction and cavitation, several translational studies aimed at selectively blocking the activity of the collagenase MMPs failed to prevent cavitation.[63,87] MMPs likely play complex roles in immune signaling and vascular permeability within TB lesions. While a recent report suggests that inhibiting MMPs improves drug delivery during treatment in a non-necrotizing/non-cavitating pulmonary TB mouse model, its effect on vascular permeability of necrotic granulomas, and cavitation still remains to be elucidated.[88]

The identity of the biochemical effectors of ECM destruction in TB remains one of the greatest unresolved mediators of cavitation. Identifying the primary mediators of TB-driven tissue destruction in necrotic granulomas is critical for the development of specific pharmacological interventions to prevent cavitation and lung destruction without interfering with the beneficial aspects of the immune response.

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Biophysical drivers

A possible physical determinant of cavitation is proximity to an airway. Not every necrotic lesion cavitates, and this could be due to the lack of access to an airway leading to an inability to evacuate its caseous contents into the bronchial tree and form a cavity. Nagasawa *et al.* investigated the relationship between cavity size and bronchial drainage and found that larger cavities were drained by larger bronchi, suggesting that the size of the pre-cavity focus must match an appropriately sized airway for cavitation to occur.[89] Similarly, increased air pressure inside the cavity from a caseous one-way-valve between the cavity and the draining bronchus may lead to the initial cavity formation (figure 4B).[90–92] However, while a positive-pressure model may explain early cavitation events, many large cavities have open connections between the cavity-space and the draining bronchus. The preferred location of cavities in the lung apices could also have a role in cavity formation. In addition to receiving the highest relative ventilation, the apices of the human lung are also the site of the highest mechanical stress. Recently, Ihms *et al.* suggested that the pull of lung-tissue at the periphery of the cavity wall also aides during cavity formation and growth.[93]

The biophysical mechanisms of large-cavity growth remain largely unresolved and further investigations are required to evaluate tissue-level biophysical changes that could be contributing to cavity formation and subsequent enlargement. For instance, the combined influence of vascular necrosis and increased oxygen demand from immune cell influx within large granulomas may create a hypoxic environment focused at the center of a lesion which drives central necrosis in the pre-cavity nodule.[94,95]

Microbial drivers

While many infectious and non-infectious diseases can form lung cavities, TB causes an especially high rate of cavitation.[42] Since *M. tuberculosis* is dependent on aerosolization for transmission, the bacillus may have evolved specific virulence factors that promote cavitation.[96] However, there is little evidence that intrinsic molecules of *M. tuberculosis* are capable of directly mediating tissue destruction leading to cavitation. Rather, most models propose that *M. tuberculosis* causes cavities indirectly by promoting an immune response that leads to cavitation. A possible exception is the recent characterization of tuberculosis necrotizing toxin which induces host-cell necrosis.[97] There are also some suggestions of heterogeneity in the ability to cause tissue destruction among *M. tuberculosis* strains. Bacteria isolated from efficient TB spreaders can lead to more necrosis and lesions that resemble caseating granulomas when used to infect mice, compared to bacilli isolated from inefficient TB spreaders.[98]

Earlier studies by Yamamura and colleagues evaluated the cavity-forming properties of purified components of the mycobacterial cell wall by trans-thoracically injecting milligram quantities of *M. tuberculosis* directly into the lungs of rabbits.[60,61] The combined injection of *M. tuberculosis* or *M. bovis* cell wall protein mixed with a mycobacterial long branched-chain fatty acid glycolipid, such as trehalose 6,6′-dimycolate (TDM), was able to cause cavities in rabbits (figure 4C). Immunogenic proteins like ESAT-6, encoded by genes of virulent *M. tuberculosis*-complex, but lost from the BGC genome, are known to promote granuloma formation.[99] Similarly, TDM stimulates macrophages to release pro-

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inflammatory cytokines leading to fibrosis and necrosis. In one possible mechanistic model, the protein fraction leads to the acquisition of cell-mediated immunity and the recruitment of lymphocytes to drive granuloma enlargement, while the glycolipid cell-wall provides a digestion-resistant hydrophobic substrate leading to caseous necrosis.[100,101]

Immunological drivers

The innate immunity plays a major role in the host's response to *M. tuberculosis*. Macrophages are the first line of defense and their response can either control the infection or favor its development.[102] While there is extensive data describing the role of the innate immune response in pulmonary TB, there is still more work to be done to better understand its role leading to cavitation. Necrosis is associated with cavitation in many processes (e.g. squamous cell carcinoma, pyogenic lung abscess).[103] Similarly, the relative tendency toward cell necrosis over apoptosis during the inflammatory response to *M. tuberculosis* infection is a likely factor affecting cavitation.[104,105] Therefore, cell-signaling pathways that favor necrosis over apoptosis could also bias the inflammatory response toward cavity formation. While normal *M. tuberculosis*-infected mouse strains do not cavitate, C3HeB/FeJ mice have macrophages that preferentially undergo necrosis rather than apoptosis, thereby reducing their ability to control multiplication of *M. tuberculosis* and leading to the possibility of cavitation.[69,72,106]

The adaptive immune response also plays a role in cavitation.[75,105] Cavitary TB patients have decreased total $CD4^+$ lymphocytes but increased proportion of T_h2 lymphocytes compared to non-cavitary controls.[107,108] An emerging model suggests that a T_h2 cytokine, pro-fibrotic adaptive immune response predominates in cavitary TB patients (figure 4D, table S2).[104,109] Therefore, the onset of the host-protective T_{reg} response during chronic infection may be an Achilles' heel of the anti-TB immune response leading to cavity formation and disease transmission. Higher levels of TNFα, IL-6, and IL-1β measured in bronchoalveolar lavage of pulmonary TB patients correlates with cavitary disease.[110–112] Increased LTA4H and TNF-α have also been described in the cellular borders of caseous granulomas and cavities while being less abundant in non-necrotizing granulomas.[75] In humans, neutrophils may also play a major role in cavity formation.[83,113–115] An alternative hypothesis propose that severe TB is the result of a progressive immune response to mycobacterial antigens.[116–118] These hypotheses are supported experimentally in the rabbit model and epidemiologically by Comstock *et al*'s investigations associating strong tuberculin reactivity with a greater risk for severe TB.[62,119]

Mycobacterial ecology at the cavity wall

One of the most intriguing perspectives on cavitation considers the internal surface of the cavity as a biofilm.[120,121] A biofilm is a microbial community growing on a biotic or abiotic surface within a self-assembled polymeric matrix. For bacilli at the cavity surface, caseum of the cavity wall acts as a protective matrix for growth and dissemination. The outstanding concession in this model is that caseum is not strictly self-assembled, but rather results from pathogen-induced host-cell necrosis at the cavity surface. However, in many other ways, *M. tuberculosis'* niche within cavity caseum behaves as a biofilm.

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The caseum is a substrate for bacilli in different states of metabolic dormancy and active replication.[122,123] Although predominately studied *in vitro* or in non-cavitating necrotic granulomas; oxygen, and nutrient gradients throughout the NL likely control hypoxia responses, sigma factor expression, and the mycobacterial stringent response leading to a three-dimensional organization of bacteria in different metabolic and transcriptional states.[124–129] While necrotic granulomas are known to be hypoxic, the increased oxygen tension within the caseum of newly formed cavities also likely activates resuscitation-promoting factors to drive bacillary replication and disease transmission.[123,130] The caseous niche also provides protection to *M. tuberculosis*. Since caseum is devoid of vascularization, drug penetration is dependent on diffusion which can lead to subinhibitory concentrations of some anti-TB compounds.[131] Moreover, caseum is a strong binding environment and further limits the availability of some drugs. Together with reduced access of the immune system to necrotic areas, these conditions promote genetic diversification and the acquisition of drug resistance, which are also hallmarks of biofilms.[9,11,132]

Bacilli present in caseum are mostly extracellular and exhibit altered cell wall biochemistry.[133] Although unproven *in vivo, M. tuberculosis* seems to preferentially grow in caseum as genetically regulated necrosis-associated extracellular clusters to enhance persistence, reduce antibiotic susceptibility, and promote transmission events.[134,135] Therefore, the ultrastructural identity of *M. tuberculosis* biofilms *in vivo* could also be clusters and cords bacilli growing within the favorable caseous environment at the inner edge of the cavity.

Many aspects of microbial ecology within the cavity remain unstudied, probably because of the difficulty in modeling TB cavities and obtaining specimens. However, with the recent advances in modeling, it is now possible to conduct detailed studies on bacterial physiology and growth-state within cavities. For instance, transcriptional and proteomic studies on bacteria in caseum may help resolve conserved metabolic pathways and gene-level regulatory networks necessary for proliferation, persistence, and transmission within necrotic debris.

Clinical management of cavitary TB

Historically, surgery has been one of the main treatment options for cavitary TB.[136,137] Surgical interventions for cavitary TB focus on methods to resect or collapse the cavities (*e.g.* artificial pneumothorax, phrenic nerve crush, extra-pleural pneumolysis, plombage, and thoracoplasty), or by resecting lung regions containing the cavity.[138] Although many of these interventions where pioneered before the introduction of TB chemotherapy, observational studies suggest that pulmonary resection combined with anti-TB chemotherapy for MDR-TB can achieve high success rates.[136,137,139] Current World Health Organization guidelines suggest using surgery for drug-resistant TB early in the course of the disease when the patient's risk of additional morbidity and mortality is low.[140] However, the lack of randomized controlled trials evaluating surgical treatments for cavitary TB limits the interpretation of these results and its widespread implementation.[138]

Antibiotic treatment recommendations for cavitary TB build upon those for non-cavitary TB. Since evidence of cavitation on the initial chest radiograph is a risk factor for relapse,

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[2,141,142] current guidelines, based on expert opinion, suggest that patients with cavitation on an initial chest radiograph and with positive cultures following two months of therapy should receive a seven-month continuation phase (total of nine months of treatment). An extended three-month continuation phase is also recommended for any patient with at least one cavity on a follow-up radiograph.[143] Finally, due to the apparent suboptimal penetration of rifapentine into the cavity wall, the option for once-weekly rifapentine dosing during continuation phase is restricted to noncavitary patients.[2,68,144,145]

Recent studies have investigated the penetration of anti-TB drugs into the cavity wall.[17,68,132] Some anti-TB drugs like pyrazinamide, distribute homogenously throughout the cavity wall with levels similar to those in plasma.[17,146] However, other drugs have limited penetration leading to impaired treatment and increased risk of developing drug-resistance.[7,132] These results suggest that drug penetration into cavitary lesions should be a consideration when selecting anti-TB drugs for clinical trials and treatment regimens should be optimized for patients with cavitary disease.

Healing of TB cavities

Between 20-50% of cavitary TB patients have persistent cavities after completion of anti-TB treatment.[147] However, the healing response is incomplete and results in fibrotic scarring which can lead to open (cavity airspace remains) or closed healing (cavity resolved to scar tissue or calcified foci).[148,149] Open healing leads to a higher risk of secondary colonization of the cavity, mostly by fungal pathogens (e.g. *Aspergillus fumigatus*), but additional research is needed to evaluate the relationship between persistent cavities and increased risk or worse disease with other co-infections.

The mechanism of cavity closure is poorly understood. Coryllos suggested that complete caseous occlusion of the draining bronchus resulted in cavity collapse followed by fibrotic scarring due to atelectasis and reduced partial oxygen pressure within the cavity, hindering the growth of *M. tuberculosis*.[91,92] Recently, Corbetta *et al.* used surgically implanted endobronchial one-way valves to cause regional hypoventilation with subsequent improvement in cavitary disease.[150] However, additional research is needed to determine the utility of these procedures. Regeneration of functional pulmonary tissue is the ultimate goal of TB cavity treatment, but this is a challenging paradigm since the lung is composed of a complex tree of alveoli, bronchi, and blood vessels created in the context of the fetal lung.

Conclusion

Cavitary TB is a fundamental event in TB pathogenesis and a key driver of disease transmission and poor outcomes. Unfortunately, cavitation is one of the least-studied aspects of the disease. Recent advances in preclinical modeling of cavities provide valuable tools and insight toward understanding cavitary TB. However, there are still many questions that need to be answered. Novel interventions and stratified chemotherapy treatment regimens should be optimized for patients with cavitary disease. The cornerstone of these efforts must be stronger multidisciplinary collaborations aimed at reducing the worldwide burden of cavitary TB.

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Search strategy and selection criteria:

We searched PubMed and Google Scholar for articles and books, using combinations of the search terms “tuberculosis”, “cavities”, “cavity”, and “cavitary”. Results were not restricted to clinical data, were without language restrictions, and published any date before March 3, 2019. Relevant articles resulting from these searches and references cited in those articles were reviewed. Preference for inclusion in this Review was given to the latest evidence from publications within the past 10 years.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

We would like to thank Dr. Eric Nuermberger and Dr. Elizabeth Ihms for thoughtful discussions and insight into the pathogenesis of cavitary TB. We also gratefully acknowledge Marjorie Winslow Kehoe and the archival team at the Alan Mason Chesney Medical Archives of the Johns Hopkins Medical Institutions.

Funding:

Drs. Jain and Ordonez received support from the National Institutes of Health (Director’s Transformative Research Award R01-EB020539, R01-HL131829, R01-EB025985, R56-AI145435, and R21-AI149760). Dr. Bishai received support from NIH (R21-AI127311, R01-AI37856, and R01-HL133190). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Key points:

- Cavitation is a dangerous consequence of pulmonary TB associated with poor outcomes, treatment relapse, higher transmission rates, and development of drug resistance
- Modeling cavities for preclinical studies is challenging since cavities are the consequence of complex and heterogeneous host-pathogen interactions
- Recent advances in modeling TB cavities enable studies that probe the complex pathologic niche occupied by *M. tuberculosis* bacilli within the cavity wall
- Cavitation as a complex phenotype driven by biochemical, biophysical, immunological, and microbiological processes which need to be better understood to be targeted with potential therapies
- Drug penetration into cavitary lesions should be a consideration when selecting anti-TB drugs for clinical trials and treatment regimens should be optimized for patients with cavitary disease

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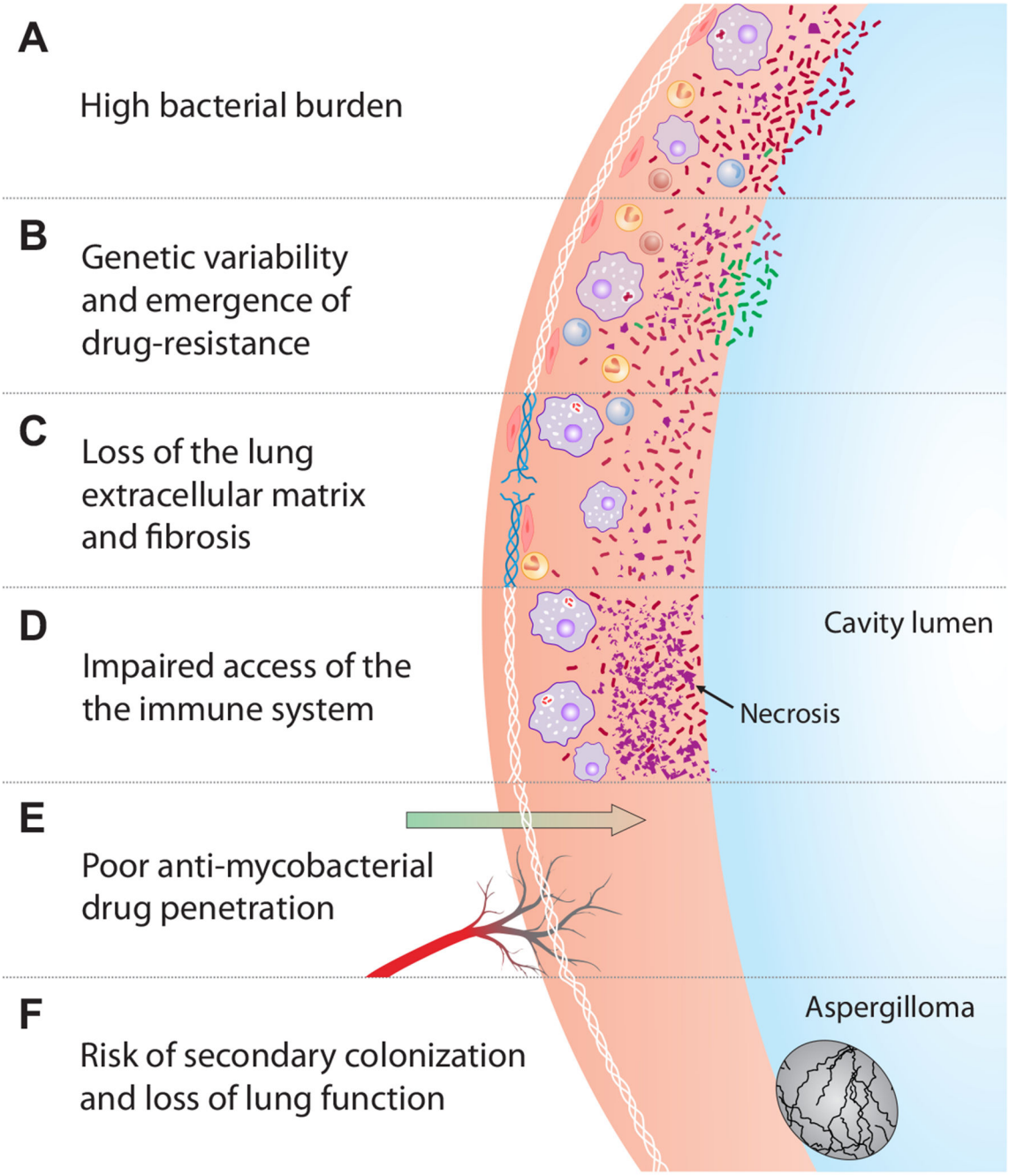

Figure 1.

Why is cavitary TB so hard to treat? (A) High concentrations of extracellular bacteria grow in the loose necrotic debris at the interior surface of the cavity.[6] (B) The bacterial proliferation leads to replication induced mutations at drug-resistance determining loci and a high probability of mutants with acquired drug resistance.[8,9] (C) Extracellular collagen matrix is depleted within caseous lesions and in the cavity wall. Depletion of extracellular collagen matrix facilitates the formation and growth of cavities since the remaining necrotic debris are easily evacuated through an adjoining bronchus. Once depleted, the healing

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response is unable to regenerate the basement membrane or lung tissue. Individuals face lifelong pulmonary deficits and a high-risk for opportunistic infections within persistent lung cavities. (D) The inner layer of the cavity wall is composed of necrotic debris. Few immune cells penetrate this region to aid in control over *M. tuberculosis* replication, and this contributes to the high bacterial burden. (E) Vascular necrosis around the cavity and strong drug-binding properties of caseum result in poor anti-mycobacterial drug penetration which also contributes to the high bacterial burden.[13,16,131] The effects of sub-optimal drug penetration may also drive selection for drug-resistant mutants.[17] (F) Cavities often persist even after they are sterilized of mycobacteria and are replaced with scar tissue (closed healing). Therefore, cavitation can lead to loss of lung volume and chronic pulmonary deficits.[19] If the cavities persist following curative therapy (open healing), then they become a vulnerable environment for secondary colonization by opportunistic infections. The combination of warm temperatures, high humidity, immune-sheltering, and lack of innate-defenses provide an opportunity for secondary colonization, often by *Aspergillus* spp.

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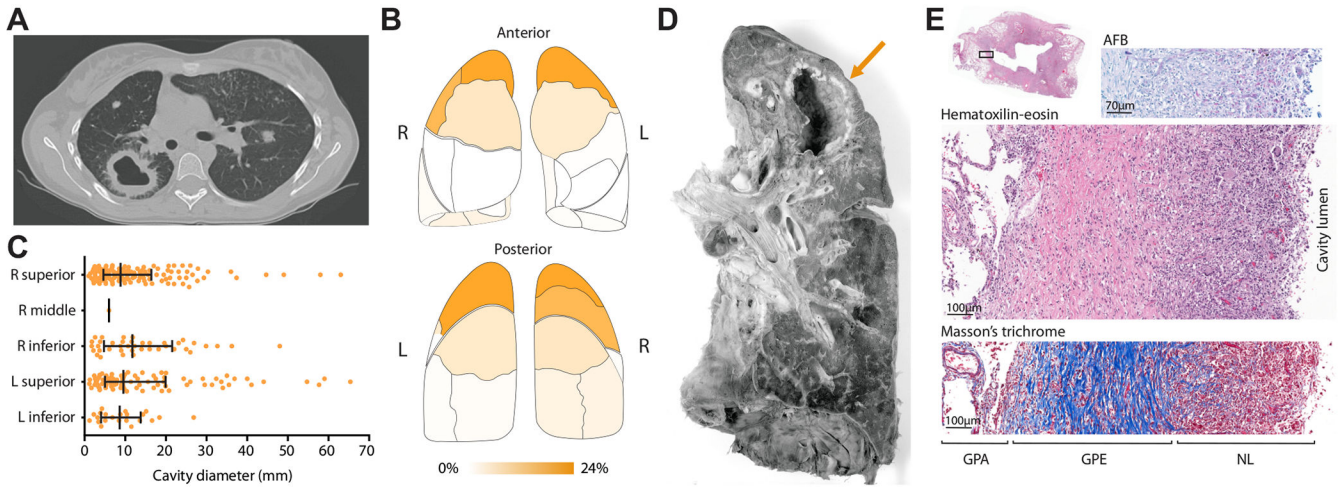

Figure 2.

The architecture of TB cavities. (A) A transverse lung field CT-scan reconstruction from a TB patient showing a large cavity. (B) The bronchopulmonary segment distribution of 287 cavities from the TB Portals Program database are represented as a heatmap of the % of total cavities evaluated. (C) Location and size of cavities analyzed in B. (D) A gross image of a large apical cavity from a TB patient. The image was obtained from the autopsy record of the Johns Hopkins Hospital and used with permission of the Johns Hopkins University Chesney Medical Archives. (E) Histology of the cavity wall of an immunocompetent human patient with pulmonary TB. Each high magnification image is a serial section from the field identified by the box in the low magnification image of the entire cavity. Data for A-C were obtained from the TB Portals, which is an open-access TB data resource supported by the National Institute of Allergy and Infectious Diseases (NIAID) Office of Cyber Infrastructure and Computational Biology (OCICB). These data were collected and submitted by members of the TB Portals Consortium. Investigators and other data contributors that originally contributed the data to the TB Portals did not participate in the design or analysis of this study.

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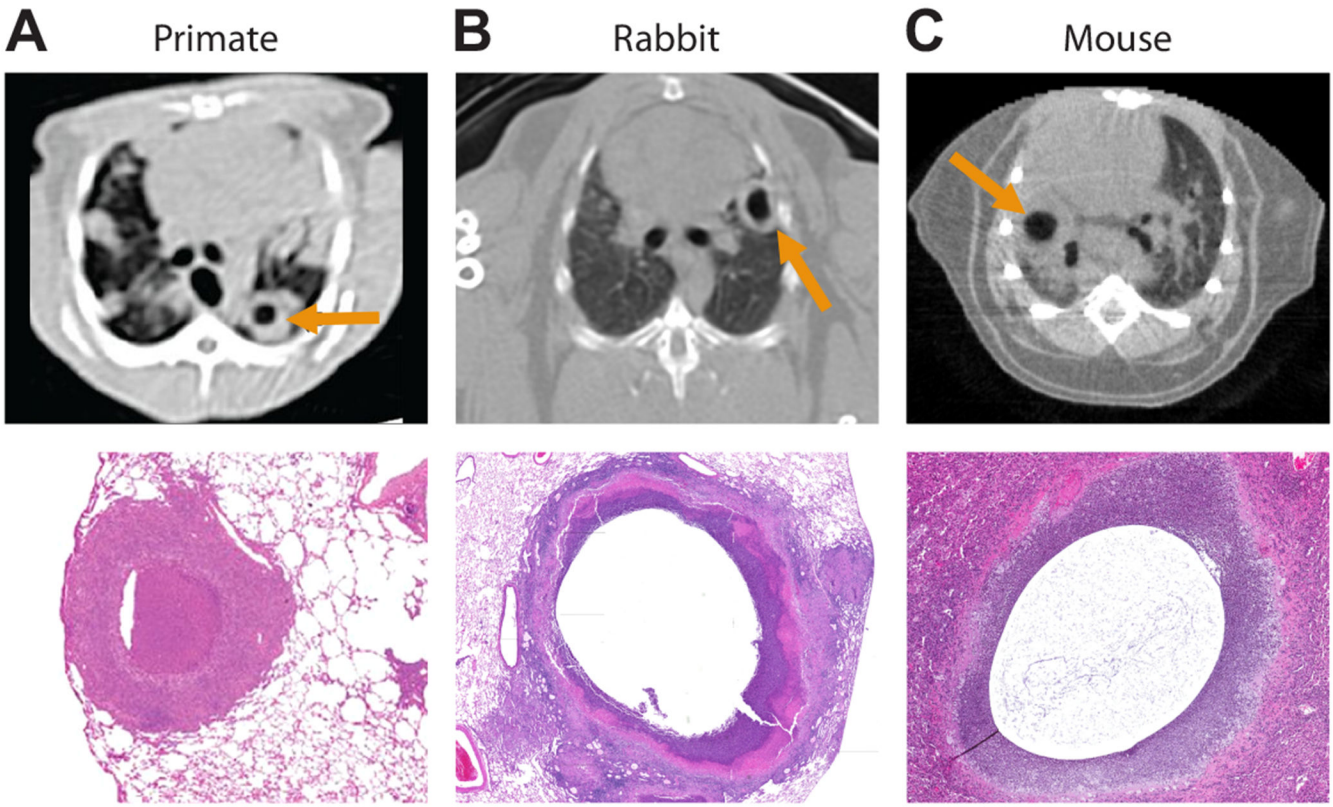

Figure 3.

Radiologic and histologic examples of cavities from cavitary TB models. Cavities in animal models infected with *M. tuberculosis* have similar radiological and histological characteristics to those found in humans. (A) Common marmoset (adapted with permission from Via *et al.*[57]) (B) New Zealand white rabbit. (C) C3HeB/FeJ mouse (adapted with permission from Ordonez *et al.*[72]).

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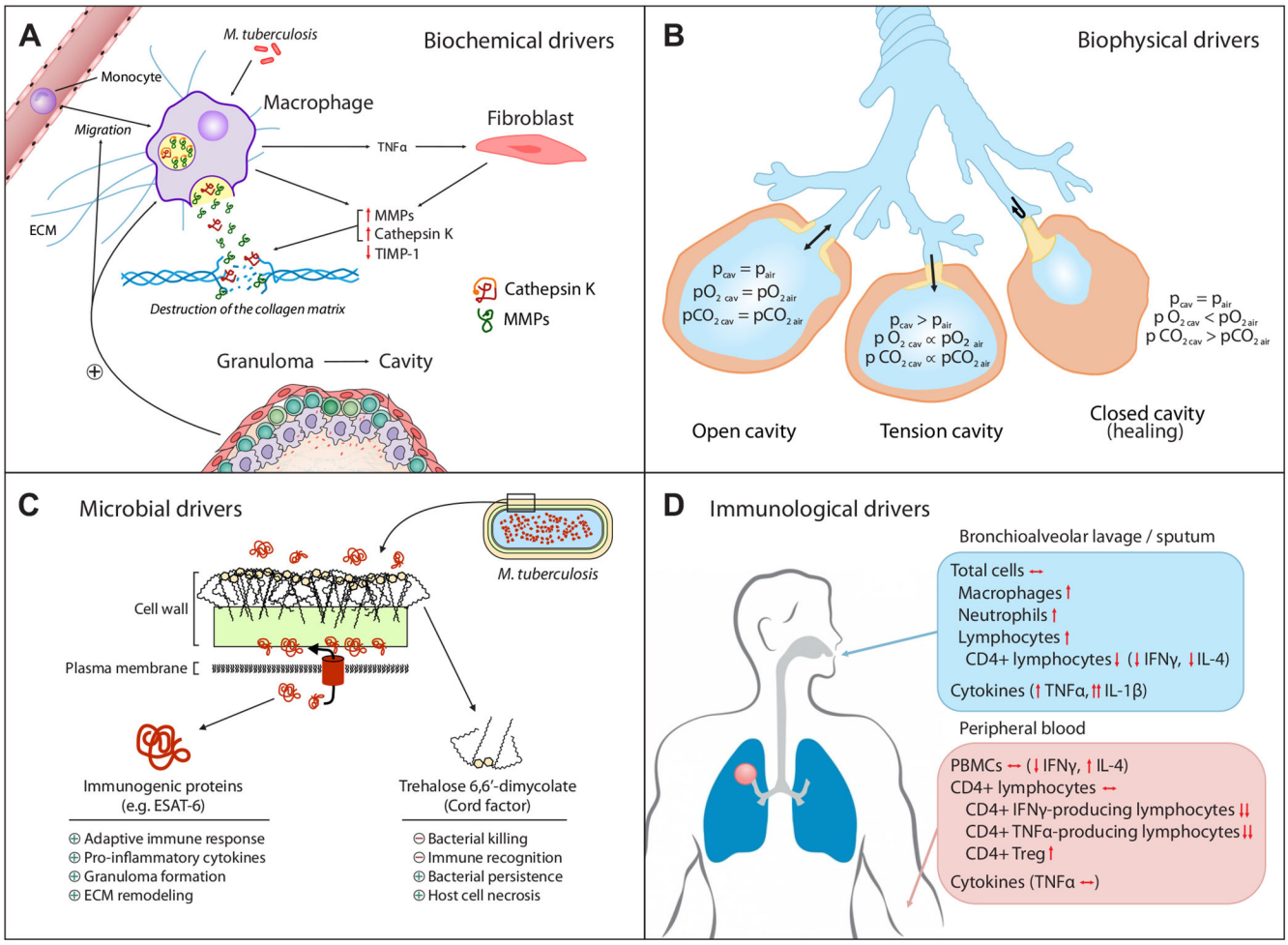

Figure 4.

Overview of the drivers of pulmonary cavitation in TB. (A) The biochemical drivers of cavitation cause basement membrane destruction and pathologic fibrosis. Black arrows indicate extracellular signaling pathways. ECM = extracellular matrix. (B) The biophysical drivers of cavitation. The prefix ‘p’ indicates the partial pressure of gases in each type of cavity compartment. Black arrows denote the bulk flow of air due to respiratory motion and/or influence by caseous occlusion (yellow material). (C) A possible model outlining the microbial drivers of cavitation. The model is based heavily on Yamamura and colleagues’ seminal work on the cavity-inducing constituents of the heat-inactivated bacillus. Figure S5 provides an overview of Yamamura’s experiments. (+) indicates that the chemical constituent has a role in increasing a given process. (−) indicates that the chemical constituent has a role in decreasing a given process. (D) The immunological profile of cavitary TB in the bronchoalveolar lavage/sputum compartment and the peripheral blood compartment. All comparisons are from studies comparing individuals with cavitary TB to individuals with non-cavitary TB. ↑ indicates an increased concentration of cells or cytokines. ↓ indicates a decreased concentration of cells or cytokines. ↔ indicates no

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difference observed between groups. Double arrows (e.g. ↑↑) indicate a strong trend. Table S2 shows the magnitude of these trends with study citations.