Cavitary pulmonary tuberculosis: The Holey Grail of disease transmission

Mark A. Yoder, Gyanu Lamichhane and William R. Bishai*

Center for Tuberculosis Research, Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, MD 21231-1001, USA

Tuberculosis retains its foothold among human populations despite increasing implementation of highly effective control measures around the world. High-burden countries would benefit from interventions that decrease disease transmission. As the majority of new cases of tuberculosis arise from patients with cavitary pulmonary disease, strategies targeting this group of patients could significantly impact disease transmission, as well as improve cure rates and decrease antimicrobial resistance. Pulmonary cavities develop late in the course of tuberculous disease, and a preponderance of the evidence suggests that the host immune response is instrumental in producing these lesions. Data from mice, rabbits and humans suggest a role for tissue-damaging enzymes released from macrophages and neutrophils and the inflammatory effects of tumour necrosis factor, interferon-*g*, interleukin-4 (IL-4), and IL-12 in cavity formation. Less well studied has been the pathogen's contribution to this process, although various mycobacterial factors, including lipoarabinomannan, ESAT-6, and cord factor, are known to directly cause or exacerbate tissue damage. A major obstacle to the study of this characteristic human lesion – the lack of a rapid and reproducible animal model – has been overcome by a recently-developed bronchoscopic rabbit model of cavitary pulmonary tuberculosis. Exploring this model with current immunology and molecular techniques is expected to yield new insights into the pathogenesis and prevention of this destructive process.

Impact of current control measures on the global burden of tuberculosis

Tuberculosis continues to be a global problem, with nearly one third of the world's population harboring latent infection[1] and an estimated 8.3 million new cases of and 1.8 million deaths attributed to this disease[2] in 2000. The incidence of new cases continues to increase[2] despite continued BCG vaccination of newborns and increased implementation of directly observed therapy (DOT) in high-burden countries. These strategies are effective at curing disease and decreasing the progression from infection to disease, and have shown promise in eradicating this infection from countries with the resources to implement both adequately. Most high-burden countries do not have such resources, however, and would likely benefit from additional strategies, in particular ones aimed at decreasing the transmission of infection to susceptible hosts. As patients harboring cavitary lung lesions are a major source of disease transmission, efforts to better understand the pathogenesis of cavitation may ultimately yield novel strategies for reducing both disease progression and transmission.

Clinical implications of cavitary pulmonary tuberculosis

Cavitary pulmonary tuberculosis has been recognized as a public health threat for many years, but additional consequences of this form of disease have been defined more recently. Clinical trials have clearly demonstrated the impact of cavitary lesions on disease transmission, response to treatment and bacterial resistance. It has been known for some time that patients with smear negative disease are able to infect others[3], but patients with cavitary lung disease are much more likely to do so[4]. In fact, patients with cavitary lung lesions are the main source of disease transmission[5]. Cavitary disease on chest radiography is associated with increased time before sputum smears and cultures convert to negative following initiation of antituberculous chemotherapy[6]. Recently, cavitary lung disease was found to be one of the independent risk factors predicting relapse following completion of six months of DOT. Baseline risk for relapse was 2%, while patients with cavitary disease had a 5% chance of this occurrence, and those with both cavitary disease and positive sputum cultures following the first two months of therapy (intensive phase) had a relapse rate of 22%[7]. It is also more likely that patients with pulmonary cavities will harbor drug-resistant organisms. Bacillary multiplication within pulmonary cavities is facilitated, perhaps due to the high oxygen tension resulting from bronchial communication[8], resulting in up to 10^{11} bacilli per gram of tissue[9]. Given that spontaneous mutations resulting in antimicrobial resistance occur at varying rates within bacterial populations, ranging from 1 in 10^4 for resistance to ethambutol to 1 in 10^8 for rifampin[10], the probability of resistant mutants emerging from cavitary lesions is high.

*For correspondence. (e-mail: wbishai@jhsph.edu)

Current model of the pathogenesis of cavitary lesions

Despite the clinical data available concerning cavitary lung disease, the pathogenic evolution of these lesions is not fully understood. Cavity formation is felt to be one of the final stages of tuberculous disease. Infection is initiated following inhalation of bacilli contained within droplet nuclei and phagocytosis by alveolar macrophages. While many of these cells are able to eradicate the ingested organisms, phagosome maturation appears to be blocked in resting, non-activated macrophages[11], permitting intracellular replication of virulent mycobacteria. The pathogens lyse these cells, only to be engulfed by a second wave of monocytes recruited from the bloodstream to the focus of infection by cytokines and chemokines. This cycle of phagocytosis, intracellular multiplication, and cell lysis is repeated, resulting in an expanding granuloma, until T cells are recruited to the site and activate infected macrophages, enabling them to kill intracellular bacilli[12]. Thereafter, the central region of the granuloma consists of necrotic and apoptotic cells, forming the caseum characteristic of tuberculous lesions[13]. Caseous material is generally felt to be a poor medium for growth of extracellular bacilli, and thus granulomas do not contain rapidly replicating organisms[14].

A key event in the pathogenesis of cavity formation is liquefaction, during which, by poorly understood mechanisms, the solid caseum liquefies and in so doing becomes a permissive environment for bacterial replication. The explosive growth of extracellular bacilli in liquefied granulomas is associated with enlargement and subsequent erosion of these lesions into blood vessels or small airways. This communication results in the evacuation of the central portion of the lesion, with potential dissemination of live bacilli into the bloodstream or bronchial tree, where secondary foci of infection may develop or the bacilli may be suspended in air currents and expelled into the environment[8,12] (Figure 1). Clearly, not all granulomas undergo liquefaction, and, of those that do, not all are permissive for bacterial growth. What determines the fate of any given granulomatous lesion is unknown and comprises an important challenge for pathogenesis research. One presumption that follows from the above pathogenic model is that all of the preceding steps must occur in order for the final lesion, the pulmonary cavity, to manifest.

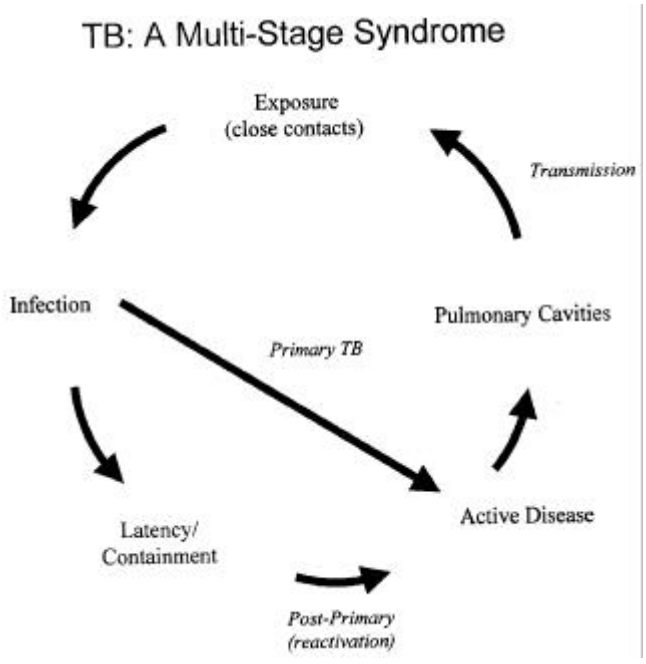

Figure 1. Representation of integral role of transmission from pulmonary cavities in the 'life cycle' of TB infection and disease within a population.

It follows, then, that the interaction between host and pathogen determines whether such lesions will occur. As resistant and susceptible hosts deal with infection differently, the immune response during granuloma evolution is key to understanding the early steps leading up to cavity formation. Macrophages lining the alveolar walls are the first components of the immune system to encounter inhaled bacilli. These cells secrete several chemokines, including RANTES, MIP-1*a*, MIP2, MCP-1, MCP-3, MCP-5 and IP10, which recruit additional inflammatory cells to sites of infection[15]; and cytokines, including interleukin-12 (IL-12), IL-15, IL-18, and tumour necrosis factor (TNF), which are involved in granuloma formation and maintenance[16,17]. IL-16 has also been found in macrophages in delayed-type hypersensitivity lesions, and is known to be chemoattractant for $CD4^+$ T cells[18]. TNF leads to upregulation of intercellular adhesion molecule-1 on vascular endothelial cells, providing an additional stimulus for lymphocyte trafficking into infectious foci. The interaction between lymphocytes and macrophages is crucial to containment of infection. $CD4^+$ T cells secrete interferon-*g* (IFN-*g*) in response to IL-12, which activates these macrophages, in the presence of 1,25-dihydroxy-vitamin D_3 ($1,25\text{-}(OH)_2D_3$), to kill intracellular bacilli. $CD8^+$ lymphocytes, on the other hand, recognize infected cells either through the interaction between the major histocompatibility complex (MHC) class I molecules bearing mycobacterial antigens and the CD3/CD8 complex, or by binding of mycobacterial lipid components, such as mycolic acid, lipoarabinomannan (LAM), phosphatidyl inositol mannoside, glucose monomycolate, and isoprenoid glycolipids, to CD1 molecules. $CD8^+$ T cells employ perforin, granulysin, or the Fas/FasL pathway to lyse these infected cells[19]. These specific cells and inflammatory mediators, due to their presence in the active lesion of tuberculosis, the granuloma, are presumably involved in subsequent steps leading to cavity formation.

Human studies on cavitary tuberculosis

Human data are relatively sparse and predominantly retrospective or observational but support the importance of host factors in the process of cavity formation. Patients with HIV infection are less likely to develop cavitary lung disease, despite being at increased risk for progression to disease following tuberculosis infection, developing disseminated or extrapulmonary disease, and having increased mortality from this disease. The probability of presenting with cavitary disease is related to the $CD4^+$ lymphocyte count, with advanced AIDS patients less likely to present with this typical chest radiographic pattern of tuberculous disease[20]. Cavitary disease is also less likely in older patients with tuberculosis, hypothesized to be a consequence of immunosenescence[21]. Other aspects of the immune response to tuberculosis that have been studied in humans include the role of vascular endothelial growth factor, neutrophils, *a*-defensins, IL-4, and the ratios of cytokines to corresponding soluble receptors. Serum levels of vascular endothelial growth factor were lower in patients with cavitary disease than others[22], suggesting that angiogenesis may help maintain tissue viability at sites of infection. A neutrophil influx has been demonstrated in bronchoalveolar lavage (BAL) fluid from patients with cavitary lung disease[23]. Patients with cavitary lung disease were found to have elevated levels of *a*-defensins, molecules released from neutrophils that have intrinsic activity against mycobacteria[24]. Patients with cavitary lesions were more likely to have an increase in TNF relative to TNF receptors (TNF-RI and TNF-RII), as well as an increased ratio of IL-1*b* relative to that of IL-1R. The above results suggest an imbalance favouring the persistent effect of inflammatory cytokines, which could potentially lead to increased tissue damage[25]. Peripheral blood lymphocytes from patients with pulmonary cavities were found to secrete more IL-4 than controls, which included patients with miliary tuberculosis, when non-specifically stimulated *ex vivo*[26]. A recent study corroborated this finding by comparing BAL fluid from patients with cavitary and non-cavitary tuberculosis, noting that $CD4^+$ lymphocytes from pulmonary segments demonstrating cavitary disease on high-resolution CT scan were Th2 polarized[27]. Two of these cytokines, TNF and IL-4, have been studied more extensively in animal models and will be covered in more detail below.

The mouse and the granuloma necrosis model

Various animal models of tuberculosis have been used to complete prospective studies on various aspects of this disease. Mice, guinea pigs, rabbits and non-human primates have been the most commonly used models, the former being perhaps the most exploited for such work due to the availability of immunologic reagents and knock-out (KO) strains. One drawback of this species is that mice do not develop cavitary disease during the course of tuberculosis infection; nevertheless, investigators from two independent labs took advantage of various KO mice and a virulent strain of *M. avium*, a non-tuberculous mycobacterium, to screen for immunologic mediators essential for the development of granulomatous necrosis, which was used as a histopathologic surrogate for cavity formation in these experiments[28,29]. They found that necrosis did not develop in mice with deletions of the *ab* T cell receptor or IFN-*g*, highlighting the importance of the interaction between $CD4^+$ lymphocytes and macrophages to this process. CD40-KO mice also did not develop necrosis. Disruption of recombinase activating gene-1 (RAG-1) results in an absence of T and B cells, and RAG-1-KO mice are thus severely immunocompromised. These mice did not demonstrate granulomatous necrosis, consistent with the hypothesis that a sequence of events, including formation of granulomatous lesions, must occur before cavities can develop. Data for TNF-KO mice were not shown, but the investigators commented that these mice similarly failed to form granulomatous lesions and had significant early mortality, which made it impossible to evaluate them in regards to the surrogate histopathologic lesion. TNFRp55-KO mice did demonstrate marked granulomatous necrosis however. Absence of *gd* T cell receptors, CD8, or *b*2-microglobulin (the last leading to deficiency of MHC class I and CD1 molecules) did not affect the development of granulomatous necrosis, and thus these components of the immune response were not felt to be essential for subsequent cavity formation. Interestingly, CD4 and MHC class II-KO mice developed some granulomatous necrosis, although less than controls, suggesting that while $CD4^+$ T cells are important to the process, other cells can subserve a similar immunopathogenic role, such as $ab^+CD4^-CD8^-$ cells bearing CD1. A role for IL-12 was also suggested, as mice deficient in the production of this cytokine did not demonstrate necrosis, although one study found that granulomas were poorly formed[29] while the other demonstrated an increased inflammatory cell influx into granulomas[28]. In both studies, IL-10-KO mice developed necrosis that was more significant than that seen in controls, suggesting a role for this cytokine in limiting tissue-damaging immune responses. Perforin deficient mice and mice overexpressing the anti-apoptotic molecule Bcl2 were found to develop granulomatous necrosis similar to wild-type mice. Conflicting data were found for nitric oxide synthase-2 (NOS2)[28,29]. One caveat in interpreting the data from these studies is that granulomas must form before necrosis can be observed, and this event did not occur in some of the strains used in these experiments, including IL-12p40-, IFN-*g*-, and RAG-1-KO or nude mice. To control for this, one study showed that depletion of $CD4^+$ cells or neutralization of

IFN-*g* or IL-12p40 in wild-type mice at a time point when granulomas had formed prevented subsequent necrosis[29].

Lessons learned from the rabbit

A major obstacle to the study of cavitary pulmonary tuberculosis has been the lack of a rapid, reproducible model of this characteristic human lesion. The full spectrum of disease seen in immunocompetent humans is most closely reproduced in rabbits, as these animals share a natural resistance to infection but manifest pulmonary cavitary lesions during the course of progressive disease. Early investigators noted that rabbits that had been immunized with heat-killed *Mycobacterium bovis* and were subsequently infected with small numbers of this virulent tuberculous mycobacterium developed cavitary lung lesions after 6–10 months[30]. Likewise, cavities developed when rabbits were reinfected with large numbers of bacilli at least five weeks after a more limited primary infection[31]. Yamamura subsequently conducted a number of experiments in which cavities were reliably produced between 30 and 60 days after injection of mycobacteria directly into the lungs of rabbits that had previously been sensitized by repeated subcutaneous injections of heat-killed bacilli[32]. He noted that cavities would form even when heat-killed bacilli, or various mycobacterial components, were injected into the lungs of previously sensitized animals, suggesting that the host immune response plays the preeminent role in this destructive process. Pathogen factors cannot be entirely discounted, however, as cavities were more likely to develop following infection with certain mycobacterial species, *M. bovis* in particular. A dose-response relation was observed, with rabbits infected with fewer than about 5×10^7 bacilli less frequently developing cavitary lesions[33]. Additional experiments showed that cavity formation could be attenuated with various immunosuppressive agents, such as 6-mercaptopurine and azathioprine[34], or desensitization with tuberculin-active peptide, a purified protein extract prepared from the Aoyama B strain of *M. tuberculosis*[35,36]. Cavities were known to develop even during the course of effective chemotherapy, with lesions present between 3 and 8 weeks following the initiation of treatment[33]. More recently, cavities were produced by aerosol infection with various doses of *M. bovis*. A dose–response relationship was again noted, and the process of cavity formation was accelerated in the high-dose group, with cavities present by five weeks after infection of non-sensitized rabbits[37].

Combining several of the above approaches has resulted in a method of generating cavities within five weeks by sensitizing rabbits over a period of 10 to 14 days followed by delivery of live bacilli into a selected area of one lung. These preliminary experiments have corroborated several of the above findings, including the dose–response relation and acceleration of the process with the use of sensitization. Cavities formed following infection with either *M. bovis* or *M. tuberculosis*, but lesions were more reliably produced with the former species (Yoder and Bishai, unpublished data, Figure 2). Potential applications for this model include: evaluation of gene expression profiles from bacilli contained within cavities, as live bacilli are used for infection and grow to high numbers in the cavities formed; screening for bacterial genes essential in virulence and cavity formation using high-throughput techniques, such as signature tagged mutagenesis[38] or transposon site hybridization[39]. Extension of the Yamamura model of rabbit cavitary tuberculosis may also further studies on the role of various cytokines in the process of cavity formation, including IL-4, IL-10, IFN-*g*, and TNF, for which antibodies or nucleotide sequence data in the rabbit are available. Additionally, it offers the potential to assess the relative contributions of apoptosis and necrosis to the cell death observed in histopathologic specimens from cavitary lesions; to further investigate the role of macrophage enzymes to this process; and to test agents or immunomodulatory strategies for their ability to prevent cavity formation.

Host factors in cavity formation

As suggested above, elements of the host immune response to *M. tuberculosis* are likely to be the effectors of the tissue damage leading to cavity formation. Some

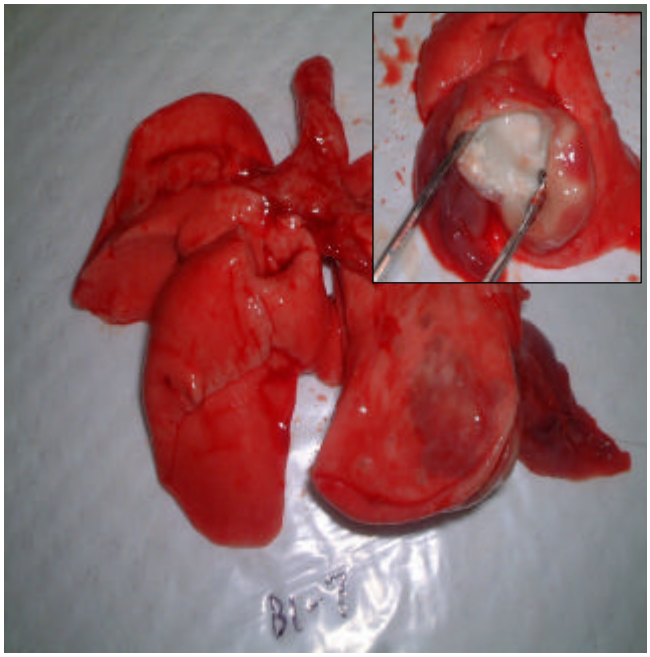

Figure 2. Lungs from a pre-sensitized rabbit six weeks following bronchoscopic infection of the left lower lobe with 10^4 colony forming units of *M. bovis*. (Inset) Caseous centre of sectioned left lower lobe cavity.

evidence supporting the role of various components of the immune system has been presented above, and Table 1 summarizes these and other hypotheses linking the host response to tissue damage. A more detailed description of the data supporting each hypothesis follows.

It has been known for some time that necrosis is a necessary step preceding cavity formation, but that lesions exhibiting this change do not invariably proceed to liquefy and cavitate. Furthermore, while lymphocytes are essential for granuloma formation, the final steps in the process of cavitation may be mediated by macrophages, specifically by dysregulated release of tissue-damaging enzymes contained by these cells. A number of such enzymes, including proteinase (cathepsin D), DNAase, RNAase, hyaluronidase, acid phosphatase, *b*-galactosidase, *b*-glucuronidase, esterase, succinic dehydrogenase and cytochrome oxidase, were abundant in macrophages surrounding caseous lesions but absent from those in areas of liquefaction. Similarly, protease and nuclease activity were found to be high in granulomas, intermediate in early necrotic lesions, and low in liquefied lesions, and lipases were detected in macrophages and epithelioid cells in pulmonary lesions but not in caseous lesions[40]. The presence of these toxic substances in early lesions suggests a role for them in the progressive tissue damage that occurs in some granulomas. *In vitro* studies have demonstrated that mycobacteria and LAM are able to induce the secretion of specific matrix metalloproteinases, in particular matrix metalloproteinase-1 (MMP-1) and MMP-9, from macrophages, and result in an increase in the MMP to tissue inhibitor of metalloproteinase (TIMP) ratio[41]. In support of these *in vitro* data, MMP-9 was also found to be more abundant in BAL fluid from patients with cavitary lung disease and cerebrospinal fluid from patients with tuberculous meningitis than controls[42].

Another intriguing hypothesis is that necrosis may result from increased tissue sensitivity to TNF. This would again support a central role for macrophages in the process, as these cells are the most abundant source of TNF within granulomatous lesions. It is known that *M. tuberculosis* can gain entry to various non-antigen-presenting cells *in vitro*, including fibroblasts, epithelial cells and endothelial cells, but bacilli are not commonly found in such cells *in vivo*. One possible explanation for this is that cells infected with *M. tuberculosis* become exquisitely sensitive to the cytotoxic effects of TNF and, when present in proximity to macrophages secreting large amounts of this cytokine, may be selectively lysed. This hypersensitivity is not strictly dependent on intracellular bacilli, as a similar effect was seen following exposure of fibroblasts and a TNF-resistant cell line to culture filtrates of *M. tuberculosis*. Sensitivity to TNF was also demonstrated in mouse footpad lesions resulting from injection of mycobacteria. When TNF is injected into these lesions, hemorrhagic necrosis develops, in contrast to mouse footpads injected with TNF alone, which demonstrates only inflammation. This necrotic response in the mouse footpad could be attenuated by depletion of complement or administration of a platelet activating factor inhibitor or misoprostol, a prostaglandin analogue[43]. One further observation suggesting a central role for TNF in tissue damage is that tuberculous lesions in patients treated with the 1,25-$(OH)_2D_3$ analogue calciferol, which is known to induce TNF secretion from macrophages, underwent liquefaction[44], and this interaction may explain the initial worsening followed by improvement seen in patients with lupus vulgaris (e.g. cutaneous tuberculosis) treated with this agent[45]. Evidence against TNF as a pivotal cytokine in cavity formation comes from the fact that TNFRp55-KO mice developed granulomatous necrosis in the model cited above[29].

One potential mechanism regulating tissue sensitivity to TNF is the local Th1/Th2 balance. In general, as noted above, the immune response to *M. tuberculosis* is Th1-polarized, with production of IFN-*g* and IL-2; but a Th2 pattern, characterized by secretion of IL-4, can be seen in some instances. The typical Th1 pattern was observed in spleen cells harvested from mice injected with 10^7 or 10^8

Table 1. Evidence supporting the role of various host factors in cavity formation

Hypothesis	Evidence
Macrophage-derived enzymes (e.g. proteinases, nucleases, lipases)	Abundant in granulomas, but absent from areas of liquefaction[40]
MMP–TIMP imbalance	Shifted in favour of MMPs in cavitary lesions[41]
Increased cellular sensitivity to TNF	Various cell lines were lysed by exposure to TNF when containing intracellular bacilli or exposed to culture filtrate[43]
Increased tissue sensitivity to TNF	Necrosis resulted from injection of TNF in combination with mycobacteria into mouse footpads[43]
Direct role of TNF	Calciferol, which induces TNF secretion, leads to liquefaction of tuberculous pulmonary[44] or cutaneous[45] lesions
Th2-predominant response	IL-4 and other Th2 cytokines predominate in lepromatous leprosy lesions (multibacillary, akin to situation in tuberculous cavity), while IFN-*g* and other Th1 cytokines are found in high concentrations in tuberculoid lesions (paucibacillary)[51]
Neutrophils	Neutrophil predominance in necrotic granulomatous lesions from NOS-deficient mice[53]

bacilli, while these cells became Th2 polarized following injection of 10^9 organisms[46]. A shift in the IL-2 to IL-4 ratio was observed during experimental pulmonary tuberculosis infection in mice. This shift occurred by 60 days after initiation of infection in all areas of the lung, and also noted at this time point was a predominance of $CD8^+$ T cells over $CD4^+$ cells[47]. A late Th2 switch was also observed in a study evaluating $CD4^+$ T cell cytokine secretion in response to incubation with macrophages pulsed with live or dead mycobacteria, secreted proteins, or the mycobacterial heat shock protein hsp60. Interestingly, IL-4 secretion was not apparent following incubation of lymphocytes with macrophages infected with live mycobacteria[48], suggesting that Th1/Th2 balance may be dependent on the predominant antigens, which may change over the course of infection.

Further evidence for a role of the Th2 pattern and $CD8^+$ lymphocytes in tissue damage comes from diseases other than tuberculosis. For example, a comparison of cytokine profiles from tuberculoid (resistant) and lepromatous (susceptible) leprosy lesions showed that the CD4 to CD8 ratio favoured the former in tuberculoid lesions, while the ratio was reversed in lepromatous lesions[49,50]. Furthermore, macrophage-derived IL-1*b*, TNF, GM-CSF, TGF-*b*1, and IL-6 and the lymphokines IL-2, IFN-*g* and LT predominated in resistant lesions, while IL-4, IL-5 and IL-10 were more abundant in lepromatous lesions. Additionally, $CD4^+$ cells producing IL-2 and IFN-*g* adoptively transfer resistance to leishmaniasis, while those producing IL-4 and IL-5 accelerate lesion formation[51]. IL-4 was also found to affect the character of granulomas formed in response to purified protein derivative. While these lesions did not demonstrate necrosis, an increased eosinophilic infiltrate and elevated levels of procollagen type III were thought to be indicative of a tissue-damaging inflammatory response[52]. Overall, this evidence suggests that T cells determine the degree of tissue destruction in tuberculous lesions, in that the Th1/Th2 balance modulates the toxicity of macrophage-derived TNF.

Another component of the immune system that may be playing a role in tissue damage but is often overlooked in the context of tuberculous infection is the neutrophil. Mice deficient in NOS2 were found to form granulomas that were infiltrated predominantly with neutrophils, and these lesions had a greater component of necrosis than those from wild-type mice. The authors hypothesize that the increased tissue destruction may have resulted from the loss of the inhibitory activity of NO on the immune response[53]. This result contradicts the one found with NOS2–KO mice infected with *M. avium* described above[29]. Indirect evidence for a negative role of neutrophils in tuberculous infection comes from a study showing that neutrophils are more predominant in lesions of *gd* T cell–KO mice, and this strain demonstrated increased mortality following high-dose infection with *M. tuberculosis*[54].

Bacterial factors

Clearly, the host immune response to tuberculosis is important in the development of pulmonary cavities, but pathogen factors also likely contribute to this process. A genome-wide search for genes in *M. tuberculosis* that could be essential for a cavity-forming phenotype was conducted by comparing chest radiographs from patients with active tuberculosis with the clinical strain of *M. tuberculosis* isolated from each patient. Strains with large regions of deletion were less likely to be associated with cavitary disease. The authors searched these regions for specific gene deletions, and postulated that the following genes, by virtue of their known or presumed role in virulence, could contribute to cavity formation: *pks5*, four phospholipase C genes, and several transcriptional regulators and membrane proteins[55]. A study utilizing *in situ* hybridization on *M. tuberculosis*-infected human lung specimens containing various types of lesions found that gene expression in necrotic regions of granulomas differed from that in non-necrotic areas of the same granuloma or non-necrotic granulomas[56]. Recently, ESAT-6, a secreted protein encoded by a gene contained within the region of deletion 1 of BCG, was shown to induce necrosis of alveolar epithelial cell lines *in vitro*[57]. Lipoarabinomannan, a mycobacterial cell-wall component, has been shown to induce TNF and IL-1 secretion from macrophages[58], suggesting a role for this specific antigen in triggering a putative tissue-damaging cascade. Glutamate synthetase and alpha antigen are other mycobacterial proteins known to induce TNF secretion[59]. Cord factor, trehalose-6,6′-dimycolate, is a mycobacterial component that has been shown to have toxic effects in animal models[60]. Furthermore, several single gene-disrupted strains of *M. tuberculosis* demonstrate the ability to multiply and persist to the same degree as the wild-type strain in a mouse aerosol infection model, but result in fewer histopathologic changes and improved survival in time-to-death analyses[61,62].

Conclusions and future prospects

The data presented above represent nearly 50 years of investigation, and the recent recognition of the clinical importance of cavitary pulmonary tuberculosis has revived an interest in unravelling the mystery of the mechanisms underying this destructive pathologic process. Some of the hypotheses above have a good experimental base despite the relatively scant literature in this area. Several converging lines of evidence have suggested a central role for TNF. This cytokine is also strongly linked to granuloma formation, and thus knockout mice do not develop the histopathologic lesions that necessarily precede necrosis and cavity formation. The hypothesis of increased tissue sensitivity to TNF, augmented by a Th2-

dominant immune response late in infection, is intriguing, and may explain some results from earlier work. In particular, induction of IL-4 by dead mycobacteria or hsp60 may underlie the ability of heat-killed bacilli or mycobacterial extracts to cause cavitary lesions in rabbits, and the lower threshold observed in these experiments may have been more a function of the immune response, Th1 or Th2, elicited by increasing infecting doses than the local antigen burden. Furthermore, the attenuation in histopathology and mortality seen with some gene-deletion mutants of *M. tuberculosis* suggests that pathogen factors may be more important in eliciting a tissue-damaging immune response than previously thought. The rabbit model allows for rapid and reliable production of cavitary lesions, many containing enormous numbers of bacilli. It is expected that the application of current and emerging immunologic and molecular techniques to this model will permit prospective studies of both host and pathogen factors underlying this process. Despite success in combating the spread of tuberculosis in developed countries, the disease continues to ravage populations in countries lacking the resources to implement effective control measures, and it is hoped that filling in the gaps in our knowledge of cavitary disease will permit development of specific transmission-limiting strategies.

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